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BIOLOGICAL REMOVAL OF COLLOIDAL MATTER FROM WASTEWATER

Walter J. Maier

Minnesota University

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BIOLOGICAL REMOVAL OF COLLOIDAL MATTER
FROM
WASTEWATER

by

Walter J. Maier
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Minneapolis, Minnesota 55455

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ABSTRACT

This research program was designed to provide a more basic understanding of the mechanism and rates of removal of organic materials from waste waters by biological processes. Pure compound feed materials were used with the exception of a series of tests on sewage solids obtained from the Minneapolis-St. Paul Sewage Treatment Plant by centrifugation of primary effluent. Two model reactor systems were used to measure rates of substrate removal. The film flow reactor, characterized by a stationary biological slime layer, was used to study removal kinetics under conditions where mass transfer may be a limiting factor. A well mixed batch reactor was used to study rates of removal under conditions where biological processes are rate controlling. Rates of substrate degradation and carbon removal are reported for each of the pure compounds using acclimated innoculum. Rate data from batch reactors are expressed in terms of microbial growth rate coefficients. Rate data from the film flow reactor is reported as the quantity of substrate removed per unit of slime surface per unit of time.

This report was submitted by the University of Minnesota, Minneapolis, Minnesota 55455, in fulfillment of Project No. 17030 DGO under the sponsorship of the Environmental Protection Agency.

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SUMMARY

This research program was designed to provide a more basic understanding of the mechanism and rates of removal of organic materials from waste waters by biological processes. Such information is needed to develop fundamentally sound process variables correlations and to stimulate development of new design concepts or process improvements of existing biological treatment devices.

Typical municipal waste waters contain a wide variety of organic materials; the major categories of biological compounds are represented; colloidal materials include carbohydrates, proteins, and lipids; soluble constituents include sugars, amino acids, and fatty acids. Soluble constituents typically account for less than 25 wt.% of the organic fraction. It follows that degradation of colloidal and larger particulates is a crucial, if not controlling factor, in biological treatment.

Pure compound feed materials were used with the exception of a series of tests on sewage solids obtained from the Minneapolis-St. Paul Sewage Treatment Plant by centrifugation of primary effluent. Starch and glucose were used as representative carbohydrates; peptone, glutamate, and glycine were used as representative protein related materials and palmitate and laurate were used as representative lipids. Each substrate was tested individually and in admixture with other substrates to test for interactions. Two model reactor systems were used to measure rates of substrate removal. The film flow reactor, characterized by a stationary biological slime layer, was used to study removal kinetics under conditions where mass transfer may be a limiting factor. A well mixed batch reactor was used to study rates of removal under conditions where biological processes are rate controlling; the well mixed batch reactor approximates process conditions encountered in completely mixed activated sludge type treatment devices. Biologically active sludge (innoculum) was obtained from continuous propagators, starting with sludge obtained from the Minneapolis-St. Paul Sewage Treatment Plant. In the film flow reactor, liquid medium flows over a stationary slime layer in a thin film. Flow is parallel to the slime surface so that direct contact between feed solution and cell mass is limited to the interface. Mass transfer of the substrate to the biologically active slime surface is slow, primarily by molecular diffusion; the film flow reactor simulates some conditions encountered in trickling filter processes.

Rates of substrate degradation and carbon removal are reported for each of the pure compounds using acclimated innoculum. Rate data from batch reactors are expressed in terms of microbial growth rate coefficients; pure compound substrates show growth rates in the range 0.005-0.010 per minute on acclimated innoculum. The rates of degradation of colloidal substrates cover a broader range, presumably because hydrolytic cleavage, which is mediated by exoenzymes, precedes the final utilization of the substrate, and may be a limiting factor if

the microbial population is not acclimated to the colloidal substrate. Data, using starch substrates, show that enzyme activity decreases as the reaction proceeds. It appears that the hydrolytic exoenzymes are inhibited by the products of hydrolysis; products of hydrolysis act as competitive inhibitors as evidenced by the increase in the calculated values of Michealis-Menton adsorption coefficients.

Substrate uptake of slime surfaces acclimated to single substrates seem to be mass transfer limited at concentrations below 100 mg/l and reaction limited at higher concentrations. Rate data from the film flow reactor are reported as the quantity of substrate removed per unit of slime surface per unit of time, (mg/cm^2-min). Maximum uptake with single substrates is in the range 50-250 x 10^{-6} mg/cm^2-min. Highest rates were observed with lauric acid, glucose, and when mixed substrates were used. Lowest uptake rates were observed with starch feed; starch-carbon uptake was only 50 x 10^{-6} mg/cm^2-min. even though the rate of starch degradation was 2-4 fold greater. By contrast, protein removal and carbon disappearance are more nearly equivalent. Both starch and protein molecules are far too large to be absorbed directly into bacterial cells, leading to the conclusion that exoenzymes, either free or bound to the cell surface, are responsible for the hydrolytic breakdown of colloids, followed by ingestion of soluble fragments. The starch-carbon data suggest that the sites for hydrolysis and ingestion are independent and physically separated, as evidenced by the low carbon uptake rate.

Acclimation of the slime surface is necessary when new substrates are introduced; glucose is an exception. Acclimated surfaces may loose their activity for metabolizing a given substrate if the substrate is removed for any appreciable time, (a matter of hours). Acclimation of the surface to an entirely new substrate may take several days. It appears that an entirely different surface coating (different bacterial species) is formed.

Use of mixed substrates generally results in higher rates of carbon uptake. This is particularly pronounced when carbohydrates are added. Preferential utilization of glucose in pure compound mixtures and lactose from skim milk substrated have been observed. Nevertheless, there is concurrent utilization of other substrated e.g. protein and starch degradation as well as some uptake of carbon from noncarbohydrate sources.

The results of this study provide new insight on the behavior of biologically active slime layers and provide quantitative information on rates of degradation of the major biochemical constituents of waste water. The results have been used to guide pilot plant test programs sponsored by the University of Minnesota for the development of more fundamentally based engineering design correlations. A three vessel prototype activated sludge unit with sludge recycle was used to study the effects of residence time and substrate concentration using the same types of feed substrate mixtures as used in the model reactor test program. The results will be published in the near future.

Data from the film flow reactor indicate that mass transfer may be the rate limiting factor at low substrate concentrations. This suggests that some commonly used operating modes for trickling filters may not be sound and should be reconsidered. For example, the use of effluent recirculation would appear to be undesirable at low BOD concentrations because removal may well be mass transfer limited. The use of packing which minimizes liquid hold up seems undesirable in a mass transfer limited reactor. It is therefore recommended that trickling filter plant tests be carried out to determine the effects of these process variables under closely controlled conditions. In view of the high potential rates of biological activity that are associated with the fixed slime layer, further work is recommended to delineate optimal conditions leading to new design of packing as well as the use of movable surfaces.

INTRODUCTION

Biological degradation of waste matter is a naturally occurring process, albeit a slow one, which functions in all receiving waters such as lakes and rivers. However, the capacity of this natural process of purification is limited. Overloading of receiving waters results in gross pollution and makes waters undesirable for reuse. To avoid this situation, treatment of wastewaters prior to discharge becomes increasingly important. In fact, progressively more treatment will be required in the future in order to keep pace with the rapidly increasing quantities of wastes being disposed.

Man-made treatment devices such as activated sludge units and trickling filters essentially are modifications of the naturally occurring processes. The same biological phenomenon of microbial metabolism underlies the removal of waste materials. Man-made devices differ from the naturally occurring purification process in that they are designed for relatively short detention times in order to make them economically feasible. However, increasing the degree of treatment as measured by the percentage removal of waste matter requires longer detention times and increases the costs markedly. The cost increases exponentially with percent removal. It follows that the increasing need for more complete treatment of wastewaters which is projected for the future will stimulate a search for cost reduction in the design of treatment facilities.

Design of conventional treatment facilities is based on an impressive accumulation of many years of practical experience. Within the context of these correlations, many process design improvements have been developed and cost reductions achieved. However, it seems unlikely that any major design improvements or cost reduction for convential treatment facilities will be forthcoming from this approach in the future.

An alternate approach is to develop a more fundamental understanding of the underlying principles of biological treatment in the hope of arriving at new design concepts or uncovering new ideas.

To this end, there is need for descriptions of the mechanism of biological purification at the microscopic and/or molecular level. Description at the microscopic level must consider such factors as:

Size and composition of the waste material, e.g., solutes and colloids, carbohydrates, proteins, and lipids.

Characterization of the microorganism population and its physical distribution, e.g., predominant bacterial species, mobility relative to the solution, dispersion of cells.

A measure of the rates of metabolism.

A measure of the effectiveness of mass transfer of waste matter to the cells.

It obviously is impossible to study the effects of all variables in one set of experiments. Meaningful results can be obtained only by varying a limited number of variables at one time while holding other conditions constant.

With this in mind, a broad-based model study has been carried out using a variety of soluble and collodial pure compound organic materials. The materials where chosen with a view to approximating the types of organic material that are found in municipal and industrial wastes.

The use of model reactors and pure compound substrates allows studying complex systems under controlled conditions. The models do not duplicate all aspects of the reaction system, but are designed to isolate process variable effects. This provides a more theoretical basis for correlating process results, and leads to a better understanding of the mechanisms of biological treatment.

The broad objective of this study was to investigate the biologically mediated removal of organic matter from a mechanistic standpoint in order to provide a better understanding of the overall process. Such information is needed to develop fundamentally sound process variable correlations and rate equations.

MECHANISMS AND CONTROLS OF BIOLOGICAL DEGRADATION

Biological treatment for the removal of organics from water comprises a sequence of interrelated steps including mass transfer, adsorption and a host of biochemical reactions. Considering the microbial cell as the center of this activity, the sequence starts when dissolved, or colloidal organics, salts, and oxygen from the bulk liquid phase are transported to the surface of the bacterial cell. Mass transfer is by molecular diffusion and by eddy current transport. At the cell surface, utilization proceeds with adsorption and ingestion of the nutrients necessary for growth, followed by step-wise oxidation within the cell to provide energy and building blocks for growth. By-products and oxidation products are released into the bulk liquid phase.

Two limiting situations suggest themselves--the case where mass transfer limits the rate of removal, and the case where cell metabolism limits the rate of utilization. Process variable effects will be different for these two situations because mass transfer and cell metabolism are known to respond differently to changes of such process variables as mixing, nutrient concentration, and temperature. By studing these limiting situations it is possible to learn a great deal about the mechanisms which control the overall process of removal of waste matter by biological means.

Two model reaction systems were used to simulate the above situations. A well mixed batch reactor was used to simulate conditions of high rates of mass transfer. In this situation both the fluid and the bacterial cells are free to move in all directions and relative velocities are high. This well-mixed reactor is used frequently as an idealized model of the activate sludge process.

The second model is a film flow reactor which is characterized by a stationary slime layer simulating trickling filter conditions. In this model, liquid medium flows over the slime layer in a thin film. Flow is parallel to the slime layer so that the degree of contact between liquid and cell mass is limited to the interface between the slime layer and the liquid phase. This makes for relatively low rates of mass transfer, especially if the liquid film is in laminar flow in which case mass transfer is limited to molecular diffusion. The film flow reactor thus provides a simulation of conditions where mass transfer could be the rate limiting factor.

These reactor models were used to study the removal of soluble and colloidal substrates. Glucose was used as a typical soluble carbohydrate and starch as the corresponding colloidal carbohydrate; glutamic acid and glycine were used as representative soluble amino acids and peptone as a typical colloidal protein; lauric acid was used as a typical lipid; it is soluble at very low concentrations but behaves like a colloid at higher concentrations. The results obtained with glucose as the only carbon source have been published by the author (*San. Eng. Div., ASCE, SA4 93, 91,* 1967), and are briefly summarized as background information.

Glucose Removal in Model Reactors

Process variable studies were carried out using glucose as the only source of carbon in a liquid medium containing all other growth requirements in excess. Results indicate that glucose removal can be described analytically in terms of growth kinetics of the microorganisms for the well-mixed reactor and mass transfer of nutrients for the film flow model.

In the well mixed reactor system, the rate of removal of glucose was independent of glucose concentration (above ten mg/l) but directly proportional to the amount of active cell mass. This is consistent with the fact that high rates of mass transfer are obtained in a well-mixed reactor. Thus rate of glucose removal is determined by the rate of metabolism of the microorganism.

On the other hand the film flow reactor, in which the bacterial mass (slimelayer) remains stationary at the retaining wall while medium flows over it in a thin film, showed a marked reduction in the rate of removal as glucose feed concentration was reduced below 100 mg/l. In this situation the quantity of bacterial mass as measured by slime thickness had no effect on the rate of removal. These results are consistent with the fact that the film flow reactor is a mass transfer limited system because mass transport of nutrients from the liquid film to the slime layer is by molecular diffusion, which is slow.

Colloidal Substrates - Physical Characteristics

Removal of colloidal substrates could be expected to follow a similar pattern as soluble substrates except where particle size becomes a factor. Pertinent physical characteristics of water, glucose, starch, and bacterial cells are shown below.

Typical Dimensions	H_2O	Glucose	Starch	Bacterium
Molecular weight, g/mole	18	180	10^6	10^{11}*
Diam., angstrom	4	10	10^2	10^4
Diffusion coefficient, $cm^2/sec.$	---	0.6×10^{-5}	0.08×10^{-5}	---

* Weight of 6.0×10^{23} cells.

Molecular dimension have a direct effect on mass transfer rates because larger molecules diffuse more slowly as shown by the lower diffusion coefficient for starch vs. glucose. Mass transfer by molecular diffusion is proportional directly to the diffusion coefficient and the concentration gradient. It follows that mass transfer of starch is approximately eight times less than glucose under the same concentration gradient.

Molecular size also is an important factor when considering permeability of cellular membranes. Glucose molecules are sufficiently small to pass through the cell wall and semipermeable membrane (cytoplasmic membrane) which surrounds bacterial cells. However, the diameter of starch molecules is some 100 times larger and approaches cellular dimensions. As a result it is unlikely that starch molecules can be absorbed directly.

Metabolism of Colloidal Substrates

The mechansim by which microorganisms metabolize large particles or colloids centers on two possibilities. The microorganism either can evolve a mechanism for engulfing and secreting the appropriate catabolic enzymes within the cell confines, or they can excrete the necessary enzymes into the surroundings to cause the reaction sequence to proceed outside of the confines of the permeable membrane of the cell proper.

According to Pollock (The Bacteria, Vol. 4, Acad. Press, 1962), the engulfing processes or direct passage of large molecules into cells (referred to as phagocytosis and pinocytosis) generally are associated with large cells and/or higher forms of life than the Eubacteriales. The rigid cell wall of Eubacteriales would make it difficult to develop a mechanism for passage of large molecules through the cell wall, whereas higher forms (e.g., amoeba) are surrounded by an elastic membrane, free to fold and form vacuoles. Pollocks' review of some 280 references leads him to conclude that bacterial metabolism of large molecules is mediated by exoenzymes which are available outside of the semipermeable membrane (other authors perfer the term extracelluar enzymes).

The mechanisms and controls by which exoenzymes are produced and/or liberated outside of the semipermeable membrane is not understood. As a result, it is not possible to predict the influence of process variables on the kinetics of colloid degradation. Control mechanisms for exoenzyme production have been deduced for a few pure culture systems using simple substrates. The adaptive response mechanism is the control mechanism most widely mentioned. Fukumoto (Proc. Intl. Symp. Enz. Chem., Academic Press, 1958) has shown that the formation of α-amylase in a medium free of starch substrate is negligible but can be stimulated by the addition of substrate even in the absence of nitrogen. The work by Rogers (Bioch. Journ. 39, 435, 1945) on synthesis of hyaluronidase into the growth medium gives support to the concept of adaptive response in *Streptococci* but suggest that other influences can become predominant as evidenced by the need for a peptone factor to stimulate formation of hyaluronidase. If adaptive response is the controlling mechanism, then concentration of substrate should be an important parameter in the description of the kinetics of colloid degradation.

Nomura (*Bioch. Journ.* *43*, 841, 1956) has explained the control mechanism in terms of the concept of competitive synthesis. The cell protein in synthesized preferentially and, in the absence of adequate nutrients, synthesis of cell protein stops and amylase formation preceeds. If competitive synthesis is the controlling factor, then the effects of colloid concentration would be secondary and the concentration of competing substrate solutes would be the most important parameter.

The work of Reynolds (*Jour. Gen. Microbio.*, Britl, *11*, 150, 1954) on chitinase activity is interesting particularly because the results show that agitation of the medium and culture resulted in increased chitinase activity. This suggests that mass transfer is a limiting factor in certain colloidal systems and must be considered.

The present state of knowledge of metabolism of colloids can be summarized by explaining that there are various hypotheses which describe specific observations but there is no generally applicable theory. The available information does not permit making apriori predictions about the kinetics of colloid removal in the systems of interest in waste water treatment.

The first phase of the experimental work therefore was designed to study the effects of colloid concentration. Measurements were designed to establish the relative rates of colloid degradation and utilization of the degradation products using starch as a representative colloid. Subsequent tests were carried out in the presence of other substrates to establish whether there is a competitive effect. Five different starches were evaluated. Protein and fatty acid degradation was also studied.

Kinetics - Growth and Enzymes

The underlying process in biological treatment of waste waters is the metabolic activity of microorganisms which utilize the organic materials as food for growth. Oxidizable substrates such as carbohydrates, lipids and proteins serve as a source of carbon and energy. Inorganic substrates such as NH_4^+ and H_2S can also serve as sources of energy for a few species.

Microbial growth also requires a source of nitrogen, oxygen (aerobes), sulfur, phosphorous and a broad range of inorganic salts in low concentrations. It may be assumed that all the required salts are present in excess in municipal waste waters. However, nitrogen and phosphorous may be deficient in certain industrial wastes.

Given a system where all nutritional requirements are available in excess, microbial growth will generally proceed rapidly until eventually one of the required nutrients is depleted. For this type of growth situation the rate of growth can be described by:

$$(1) \quad \frac{dN}{dt} = \mu N$$

Where N = cell mass concentration
μ = growth rate coefficient
t = time

The growth rate coefficient has a unique maximum value for each species; it is temperature dependent, affected by pH, and substrate concentration. The effect of substrate concentration has been described by Monod and can be represented by the equation:

$$(2) \quad \mu = \mu \max \frac{S}{K_c + S}$$

Where S = substrate concentration
K_c = adsorption coefficient
$\mu \max$ = maximum growth rate coefficient for the environmental conditions.

The adsorption coefficient has the units of substrate concentration and takes the numerical value of the concentration where the rate of growth is one half its maximum value. Substitution of equation (2) into (1) leads to the generalized growth rate equation which now has two dependent variables, S and N both of which are time dependent.

$$(3) \quad \frac{dN}{dt} = \mu \max \frac{S}{K_c + S} N$$

One dependent variable can be eliminated by using the relationship between yield of cell mass and amount of substrate consumed.

The relationship between cell mass synthesis and substrate utilization has been the subject of extensive investigations with pure cultures as well as with mixed cultures. In theory, there is a unique relationship between synthesis of cell mass and the substrate consumption which is defined analytically by equation (4).

$$(4) \quad \frac{dN}{dt} = -Y \frac{dS}{dt}$$

Where Y = yield coefficient, weight of dry cell mass produced per unit weight of substrate consumed.

The yield concept follows from the hypothesis that synthesis is the net result of a series of biochemical reactions (metabolic pathways) which produce protoplasm and release energy from the oxidation of substrate. As long as the same metabolic pathways are involved, the yield of cell mass per unit weight of substrate has a constant value. In practice, this condition is at best an approximation, which is acceptable as long as the changes in environmental conditions are not extreme. Assuming the yield coefficient to be constant, equation (4) can be integrated between limits to give:

$$(5) \quad N - N_o = -Y(S - S_o)$$

Where N_o and S_o are the initial concentrations of cell mass ans substrate respectively. Using the yield relationship to eliminate the dependent variable N from equation (3) gives:

$$(6) \quad -\frac{dS}{dt} = \mu \max \frac{S}{K_c + S}\left(S_o - S + \frac{N_o}{Y}\right)$$

A similar equation can be obtained in terms of cell mass, N. Equation (6) is readily integrated for K_c and Y constants, and gives:

$$(7) \quad \ln \frac{S + B}{S_o + B} + K_c \ln\left\{\frac{S(S_o + B)}{S_o(S + B)}\right\} = \mu \max t$$

Where ln = natural logarithm

$$B = -\frac{N_o}{Y} - S_o$$

Equation (7) describes substrate concentration as a function of time in terms of the maximum growth rate coefficient, the yield coefficient and the adsorption coefficient K_c as parameters. For the special case where K_c is negligibly small, i.e., at high substrate concentrations, equation (7) reduces to the form:

$$(8) \quad \ln \frac{S + B}{S_o + B} = \mu \max t$$

Either of the above equations can be used to predict the course of substrate depletion in a well-mixed batch reactor. As a rule, values of N_o and S_o can be obtained by direct measurement. The yield coefficient, Y, can be measured directly by harvesting the cell mass and simultaneous measurement of substrate depletion.

The maximum growth rate coefficient μ max may be evaluated from data obtained at relatively high substrate concentrations using equation (8); alternatively both K_c and μ max can be evaluated by trial and error from two independent sets of observations of substrate depletion as a function of time.

Data on the enzymatic degradation of colloids was analyzed in terms of a simple model where the rate of hydrolysis is defined by equation (9):

$$(9) - \frac{dC}{dt} = k_1 (E)$$

Where C = concentration of starch
t = time, min.
K_1 = rate constant
E = enzyme concentration.

Concentration of enzyme is assumed to be directly proportional to cell mass concentration, $E \sim N$. Substituting into Equation (9):

$$(10) - \frac{dC}{dt} = kN$$

Where k incorporates a proportionality constant which relates enzyme concentration and cell mass concentration.

The concentration of cell mass, N, can be replaced by the growth rate equation which relates cell mass at any time, t, to the initial concentration N_o and the growth rate coefficient μ max as shown in equation (11):

$$(11) \quad N = N_o \, [e^{\mu_{max} t}]$$

Equation (11) applies as long as μ max remains constant (logarithmic growth phase)

$$(12) \quad - \frac{dC}{dt} = kN_o \, [e^{\mu_{max} t}]$$

Integrating between limits,

$$(13) \quad C_o - C = \frac{kN_o}{\mu_{max}} \, [e^{\mu_{max} t} - 1]$$

Mathematical Analysis of Film Flow Reactor

From a theoretical viewpoint, the film flow reactor has three advantages. (i) It holds the biologically active mass stationary and thus permits the making of rapid changes in environmental conditions such as composition of the growth medium and concentration of substrate. (ii) It allows for instantaneous control of the flow of nutrients. (iii) It allows regulating contact time between the bacterial mass and the medium, independent of flow rates.

The disadvantages stem from the relatively low rates of mass transfer which limit removal of nutrients at low concentrations of nutrient. It has been shown from considerations of Reynolds number that the flow on a flat, incline plane is in the laminar flow regime. Laminar flow can also be demonstrated by the use of dye injections which show that the flow lines are parallel to the surface of the slime layer. It follows that mass transport of nutrients from the liquid film to the slime layer is by molecular diffusion, which is relatively slow. However, the flat plate reactor has the advantage that the rate of mass transfer by molecular diffusion can be described mathematically, so that it becomes possible to distinguish between the situation where mass transfer is the rate-limiting factor. A general mathematical model which describes mass transfer and reaction at the solid surface of the inclined plane has been described by the auther (San. Eng. Div., ASCE, 93, SA4, 91, Aug. 1967) and is briefly summarized below. The mathematical model is obtained from a material balance over an infinitesimal control volume as illustrated in Figure 1.

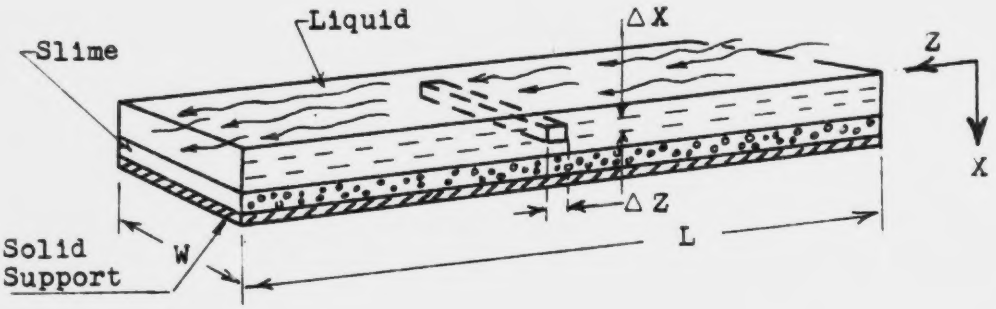


FIGURE 1

SCHEMATIC DIAGRAM OF CONTROL VOLUME

At low energy gradients the flow regime is laminar and velocity is described by equation (14):

$$(14)\quad V = \frac{\rho g \sin\beta}{2\mu}(\delta^2 - X^2) = G(\delta^2 - X^2)$$

in which V = velocity, in grams per centimeter,
ρ = density, in grams per centimeter,
g = gravitational constant, in centimeters per second squared,
μ = viscosity, in grams per centimeter-second,
β = angle in inclination,
δ = thickness of the liquid film in centimeters, and
X = distance from surface of film, in centimeters.

Let C_A represent the concentration of the limiting or potentially limiting substrate (Component A).

The material balance assumes that there is no production nor use of component A in the control volume. In effect, this means that there is no growth in the liquid film per se and that all metabolic activity takes place in the slime layer. However, the possibility of growth and use of nutrients in the liquid film was checked experimentally and found to be below detectable limits. Flux of component A in the Z direction is approximated by convective transport (equation 14). Flux in the X direction is limited to molecular diffusion. The resulting differential equation for the steady state is:

$$(15) \quad -D \frac{\partial^2 C_A}{\partial X^2} + G(\delta^2 - X^2)\frac{\partial C_A}{\partial Z} = 0.$$

Where D_A is the molecular diffusion coefficient of component A in water, cm^2/sec. Proceeding on the assumption that the surface of the slime layer is the major site for assimilation and metabolic degradation of component A, the solution of equation (15) is subject to three boundary conditions:

$$(16) \quad D_A \frac{\partial C_A}{\partial X} = 0 \text{ at } X = 0$$

$$(17) \quad -D_A \frac{\partial C_A}{\partial X} - R'' = 0 \quad \text{at } X = \delta$$

$$(18) \quad C_A = C_{AO} \qquad \text{at } Z = 0 \text{ for } 0 < X < \delta$$

The first boundary condition states that there is no mass transfer across the interface between the liquid film and air at X = 0. The second boundary condition states that the rate of transfer of component A across the interface between the liquid and slime layers at X = δ, is equal to the rate of removal of component A by assimilation at the slime surface. The term R" is a function of the metabolic activity of the microorganisms in the surface of the slime layer. Boundary condition No. 3 states that the concentration of A in the liquid film at the inlet to the plane (at Z = 0) is uniform and is represented as C_{AO}.

Boundary condition No. 2 was approximated by assuming that for active biological slime layers $R'' >>> D_A\ (\partial C_A/\partial X)$ which leads to the approximation that the concentration of component A at the interface is zero. The corresponding boundary condition No. 2 becomes

$$(19) \quad C_A \simeq 0 \quad \text{at } X = \delta.$$

Integration of equation (15) and transforming the variables into dimensionless form gives the following equation for $\bar{c}$, the vertical average concentration from the film flow reactor, at any point Z downstream from the inlet.

$$(20)\quad \bar{c}_* = 3/2 \sum_{\lambda_n} e^{-\lambda_n^2 Z*} A_n \int_0^1 (1-X_*^2)\ \psi_0(X_*,\lambda_n)dX_*$$

in which $\bar{c}_*$ = dimensionless average concentration of nutrient at any point Z_* on the plane,

λ = eigenvalue obtained as a result of boundary condition no. 2,
A = coefficient which is a function of λ and X_*,
Ψ_0 = power series in X_*.

and X_* = dimensionless distance from the surface of the liquid film, and Z_* = dimensionless distance from the inlet. (The variables have been expresses in dimensionless form to simplify the solution).

Equation (2) has been evaluated and the solutions compared with experimental results. Solutions for equation (2) are graphed in Figure 2, which relates the average concentration $\bar{c}$ (dimensionless) to the dimensionless variable Z_* defined as follows:

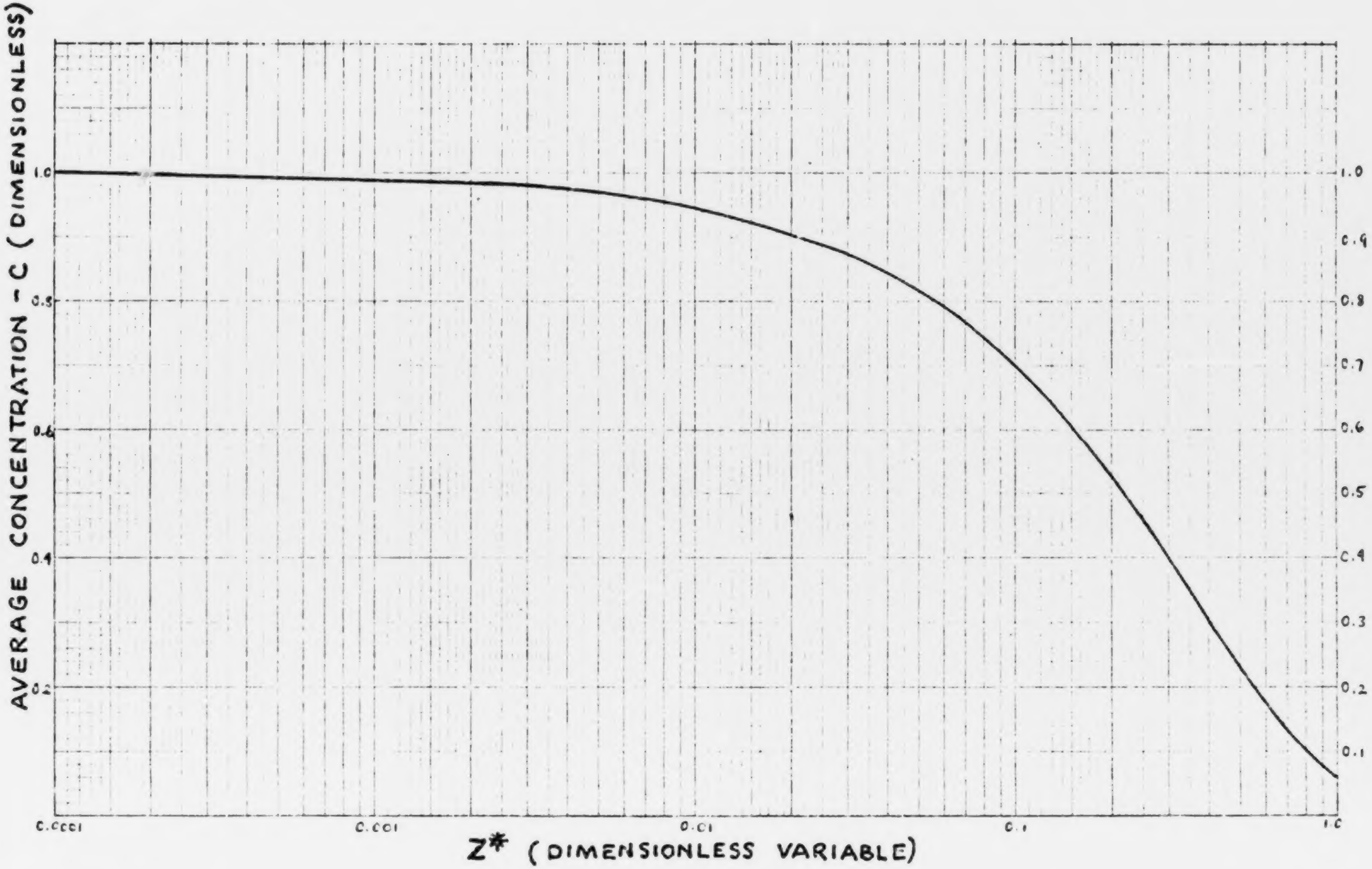
$$\bar{c}_* = \frac{C_A}{C_{AO}} \qquad X_* = \frac{y}{\delta}$$

$$Z_* = \frac{ZD_A}{G\delta^4}$$

Where C_A = average concentration any point Z, mg/l
C_{AO} = inlet concentration, mg/l
Z = distance from inlet, cm.
D_A = molecular diffusion coefficient, cm^2/sec
G = $\rho\ g \sin \beta/2\mu$ as defined by equation (14)
δ = thickness of the liquid, film, cm.

FIGURE 2

NORMALIZED SOLUTION EQUATION 20



Waste Water Composition

A thorough understanding of the composition of waste waters is essential in evaluating the effectiveness of various types of treatment and pollution effects on the environment. Several investigators have analyzed composites of municipal sewage with a view to identifying and measuring the organic constituents.

Composite samples were obtained from municipal sewage systems and subjected to physical separation methods to recover solids. The solid fractions were then analyzed for the major classes of organic materials.

Large particulate matter was removed by sedimentation for 1 hour. The supernatant from sedimentation was then subjected to centrifugation to remove a second fraction of solids. The centrifuged liquid was then passed through a candle filter to remove large colloids. (Candle filters have been used for removing bacteria from suspension. However, it is most likely that colloids such as proteins and polysacherides can pass through the candle filter). The final fraction of solids was determined by evaporation and includes both solutes and small colloids. Results are shown below.

Treatment	Amount of Solids Removed as mg/l of Carbon
1. Settled 1 hour	110
2. Centrifuged	130
3. Candle filtered	54
4. Filtrate (evaporated)	118

*Water polution Research, Report of the Water Pollution Research Board Laboratory, Stevenage, England 1959-61.

A chemical analysis of the solids from fractions 1, 2, and 3 is shown under the heading "suspended Matter". Composition of the solids from the candle filtrate is shown as "filtrate".

		Concentration as mg/l of carbon	
Constituent	Total	Suspended Solids	Candle Filtrate
Carbohydrate	77.1	21.1	56.0
Amides	2.4	2.4	
Amino Acids - Free	6.3	- - -	6.3
- Bound	70.7	63.3	7.4
Acids - soluble	17.3	6.7	10.6
- insoluble	71.6	71.6	- - -
Esters	46.9	46.9	- - -
Anionic Surfactants	9.3	2.0	7.3
Creatinine	3.0		3.0

Total Organic carbon			
- by analysis	412	294	118
- by addition	304.6	214	90.6

Inspection of the results shows that the carbohydrates are primarily solutes and small colloids. However, the bound amino acid group is primarily large colloidal or particulate matter which can be removed physically. The most noticeable group of constituents are the lipid related compounds (acids and esters) which account for more that 45% of the total organic carbon. The major portion of the lipid material is in the large colloidal and particulate fraction with only small quantities showning up in the candle filtrate.

The results obtained by Hunter (J. Water Poll. Cont. Fed., 37, 1142 Aug. 1965) show similar trends. Composite samples of sewage were subjected to successive physical separation steps, sedimentation, centrifugation and membrane filtration which are referred to as settleable, colloidal and supra-colloidal particulates respectively. Total particulate solids are approximately 80 percent organic matter, while the soluble-fraction solids are approximately 30 percent organic matter. Approximately 64 percent of the total wastewater solids is contributed by the soluble fraction, but only 40 percent of the total organic matter is contributed by this fraction.

The particulate fractions were composed largely of grease (17 percent), amino acids (19 percent), and carbohydrates (24 precent). The grease was found to be principally esterified fatty acids and unsaponifiable matter. Free fatty acids were present only in small amounts. The amino acid nitrogen content of the particulate fractions averaged only about 50 percent of the particulate organic nitrogen.

The organic compositions of the three particulate fractions were somewhat similar. The main difference was found in the considerably higher amino acid and hemicellulose contents of the supracolloidal fraction and the considerably higher cellulose content of the settleable solids.

The soluble organic matter was found to be composed largely of ethyl-ether-extractable matter, of which the organic acids were the primary constituent (56 percent). The other organic constituents present in significant quantities were the amino acids and sugars. ABS, volatile acids, phenols, cholesterol, uric acid, and creatine-creatinine were found to be minor constituents.

On the basis of these analyses, it is clear that typical sewage is a composite of a wide range of organics. All the major classes of bio chemical compounds are represented, carbohydrates, amino acids - proteins and lipids. For the purpose of this study substrates were chosen to give one or more soluble and colloidal compounds from each of the major classes of biochemical compounds.

	Soluble	Colloidal
Carbohydrates	glucose	starch
Amino Acid-protein	glycine, glutamate	peptone
Lipids	laurate	palmitate, stearate

Preparation and analytical procedures are discussed in the following section.

APPARATUS AND EXPERIMENTAL PROCEDURE.

BATCH REACTOR

Two model reactor systems were used to study the rate of biological growth and the kinetics of removal of substrate. Batch reactors were used to simulate conditions of high rates of mass transfer. The vessels were agitated by introducing air through sparging devices. Sparging provides for both rapid mixing and an excess supply of oxygen for the biological system. The sparging air was pre-saturated with water by passing it through a wet packed column. Batch reactors in a size range of 250 milliliters to 2 liters were used depending on the length of the experiment and the number of samples to be obtained. Batch reactors were used to study the effects of process variables such as temperature, concentration of substrate, type of substrate, and concentration of microbial cell mass. In any given series, all reactors were started at about the same time using aliquots of the same feed materials and innoculum. Samples were withdrawn at predetermined time intervals and immediately filtered through a 0.45 micron millipore filter to remove active cell material. Samples were then stored under refrigeration for anaylsis at a subsequent time.

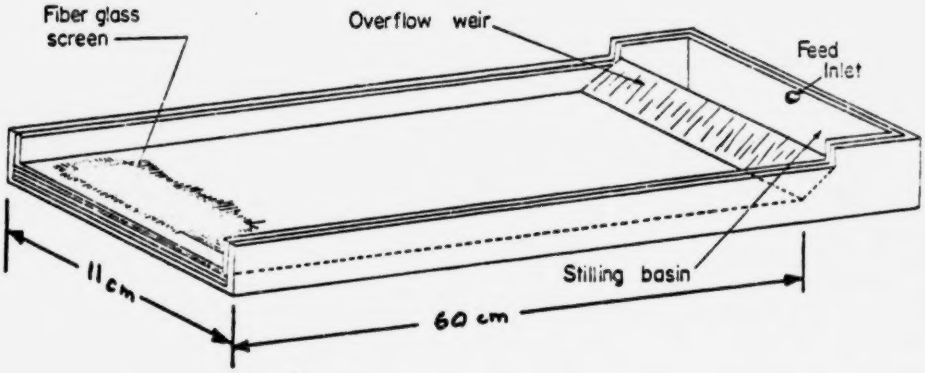
FILM FLOW REACTOR

This reactor is characterized by a stationary slime layer. Feed solution is allowed to flow over the slime layer in a thin film. Flow is parallel to the slime layer so that the degree of contact between liquid and biological film is limited to the interface between the slime layer and the liquid phase. At the conditions used in this experiment, namely relatively low flow rates, and very low hydraulic gradients, the liquid film is considered to be in the laminar flow regime. It has been shown by the author (*Journ. San Eng. Div.*, ASCE, *93*, 91, 1967) that under such conditions mass transfer is very slow because it is limited to molecular diffusion from the liquid film to the interface of the slime layer. The film flow reactor thus provides a simulation of conditions where mass transfer is, or could be, the rate limiting factor in the consumption and removal of substrate and the growth of the microbial cells.

The film flow reactor consists of a machined flat surface made of clear plastic with the dimensions shown in Figure 3. The plastic is bolted to a metal frame to prevent warping. Feed solution is introduced into a distribution or stilling basin whence it passes over a precisely machined overflow weir. Channelling and shortcircuiting through the stilling basin was observed initially, but was overcome by the use of a distribution grid. The grid consisted of wire mesh designed to provide pressure drop and thereby randomize the flow pattern through the stilling section. The outlet section was separated into three parts. For most analyses, samples were taken only from the center section which collected about 2/3 of the flow. The end sections were generally discarded because of wall effects, associated with the more rapid flow in the vicinity of the side walls. The slope of the plane was adjusted with a nut and bolt attachment at the upper end. The lower end was supported by a two point swivel support. This method of attachment facilitates leveling from side to side and allows changing the slope very easily.

The surface of the plate was covered with a fiberglass fly screen which serves as a structural framework for building up the slime layer. Growth of the slime was initiated by seeding the feed solution with effluent from a continuous propagator. After a few hours of seeding, the plane was allowed to stagnate and allow the biomass to settle into the screen. Thereafter, feed solution was continually passed over the plane and in general, growth was established within a matter of a few days. Measurements of the screen volume showed that the void spaces account for about 80% of the volume defined by the edgeview volume of the screen. After the biological materials had filled the voids, excess growth was periodically removed by scraping the surface with a knife edge. The thickness of the slime layer was thus controlled to the approximate thickness of the screen, approximately 0.05 cm. thick. Thicker slime layers were obtained by superimposing several layers of screens; 0.08 cm. for 2 screens, 0.11 cm. for 3 screens and 0.14 cm. for 4 screens.

FIGURE 3


Fiber glass screen
Overflow weir
Feed Inlet
Stilling basin
11 cm
60 cm

FILM FLOW REACTOR

CONTINUOUS PROPAGATOR

A small continuous flow, constant volume, agitated reactor was used as a source of actively growing and acclimatized microorganisms (Chemostat type system). A sparger was used to introduce air to insure high oxygen concentrations and to provide good mixing. Feed was introduced by gravity flow through a capillary tube to control flow rate. Excess was allowed to overflow to waste or collected as needed. In this type of system, liquid feed rate determines residence time and to some extent the amount of growth. It also controls the rate at which microorganisms are flushed out of the propagator and hence offers a simple method of regulating the concentration of microorganisms. The continuous propagator was originally seeded with a small sample of sewage obtained from the Minneapolis-St. Paul Sewage Treatment Plant and contained a large variety of microorganisms. The propagator was operated for several weeks, using the feed solution to be tested, in order to acclimate the microorganism population. Periodic inspection showed that a morphologically similar culture was produced within a matter of days. This is not to say that this was a pure culture, however, for all practical purposes the predominance of the acclimitised species made it behave as though it were a pure culture. The continuous propagator effluent was used as innoculum for all batch tests as well as for seeding the film flow reactor. A separate propagator was used for each new feed stock or feed solution that was tested.

FEED SOLUTIONS

Feed solutions were prepared using distilled water in a buffering salt solution. The composition of the salt solution is listed below:

Chemical Formula	Milligrams/liter
KH_2PO_4	42
K_2HPO_4	106
$Na_2HPO_4 7H_2O$	164
NH_4Cl	259
$MgSO_4$	106
$CaCl_2$	12
$FeCl_3 -- 6H_2O$	1

Reagent grade chemicals were used throughout. Soluble organic substrates consisted of glucose, glutamic acid, glycine, and lauric acid. Colloidal substrates consisted of various starches, and proteins. Fatty acids, such as lauric, palmitic, and stearic, probably behave as colloids because of their low solubility . There was some evidence of removal of fatty acids during millipore filtration, suggesting that some of the fatty acids tend to agglomerate and form miscelles which are of colloidal dimensions.

ANALYTICAL PROCEDURES

Glucose was measured using a commercially available enzyme system sold under the name of Glucostat (Worthington Chemical Co., Freehold, N.J.). This method of analysis has been used successfully by a number of investigators. Glucostat reagent contains glucose oxydase, which oxydizes glucose to gluconic acid with the release of hydrogen peroxide. The hydrogen peroxide is in turn oxidized by a chromogen in the presence of the enzyme peroxidase. The oxydized chromogen has a distinctive yellow straw color which has a strong absorption band in the 400-500 millimicron range. Color intensity was measured in a Coleman Spectrophotometer and glucose contents calculated by reference to known standards which were tested with each batch of 10-15 samples. Samples containing high concentrations of cell materials were filtered before measuring transmittance to eliminate absorption by cell material.

Carbon concentration was measured in a Beckman Total Carbon Analyzer, as described by Van Hall (Anal. Chem. 35, 315, 1963). Samples were filtered through a 0.45 micron millipore filter to remove cell material prior to carbon analysis. The carbon analyzer consists of a combustion tube followed by an infrared cell to measure the concentration of carbon dioxide produced in the combustion system. The combustion train operates at 940°C in the presence of a cobalt molybdate catalyst, and air at atmospheric pressure. The combustion gases pass through a condenser to remove water, and are then analysed for carbon dioxide in a balanced infrared cell which gives a direct readout in terms of milligrams of carbon. Samples containing more than 100mg/l of carbon were tested by injecting smaller aliquots (ten microliters), or the samples were diluted to the range of 1 to 100 milligrams per liter of carbon. The readout system covers a range from 0 to 100 milligrams per liter of carbon.

Amino acid concentration was measured using ninhydrin reagent. The procedure was patterned after that described by Yemm and Cocking (Analyst, Volume 80, March 1965, pp. 209-213). The method gives stoichiometric reactions with ninhydrin reagent for most amino acids and is both easy to use and reproducible. Ammonia does react and has to be corrected for, or eliminated from the sample prior to testing. The test procedure was calibrated using both glutamic acid and glycine. Ammonia corrections were made using direct Nesslerization to establish ammonia concentration, and then using a calibration chart of absorbance versus ammonia concentration, to incorporate corrections for specific samples. The hydrolysis products of proteins react like amino acids and give a positive reaction.

Cell mass was measured by filtering and weighing the dried solids through a 0.45 micron millipore filter. The technique is patterned after that used by Engelbrecht and McKinney (Sewage and Industrial Wastes, 28, 11, 1321, November 1956). This test provides a direct measure of the biomass and suspended solids but does not differentiate between viable cell mass and debris. The millipore filters were prewashed and predried prior to use and again weighed after drying at 103°C.

Proteins were measured using Folin-Phenol reagent. The method is patterned after that described by Lowry et al. (Journal of Biological Chem. 193, 265, 1951). The Folin reaction involves the peptide bonds of tyrosine and tryptophane in the protein. The final color is the result of two reactions, the reaction of copper with the protein in an alkaline solution, and the reduction of the phosphomolybdic--phosphotungstic reagent contained in the Folin-phenol reagent. The Folin reaction requires only 1 milliliter of sample. There are no apparent interfering substances and the determination is quite sensitive and easy to perform.

Starch contents were determined in a modified iodine test. The iodine test is described in section B of this report (Iodine Reaction and Carbon Determination of Starches).

BATCH REACTOR TEST RESULTS

Chemistry of Starch

Starch is a naturally occurring polysaccharide material consisting of glucose units jointed by α, 1, 4 linkages and to a lesser degree by α, 1, 6 linkages. Starches fall into two classes, amylase, which is beleived to be a long unbranched chain, and amylopectin which has been shown to be a branched chain structure with one terminal glucose unit for every 20 to 30 glucose residues.

Hydrolytic cleavage of starches is mediated by a variety of enzymes; amylolitic enzymes are the most common; phosphorylases and amyloglucosidases occur in special situations. The amylases fall into two categories; α amylases (α, 1, 4 glucan 4 glucanohydrolase) catalyse the scission of α, 1, 4 glucosidic bonds at random resulting in a rapid reduction in molecular weight, viscosity, and light scattering; B amylases (α, 1, 4 glucose maltose) catalyse the scission of maltose units from the non-reducing end of the chain; producing high yields of maltose but a more gradual reduction in the physical characteristics of the starch substrate. B amylase activity ceases when a branch point (α, 1, 6 bond) is reached. The resulting polysaccharide fragments that remain after B amylase hydrolysis are called dextrins or limit dextrins.

Enzymes that catalyse cleavage of the α, 1, 6 bond have been found in both intestinal bacteria and associated with certain other bacteria. However, relatively little information is available.

Characterization of Starches

Six different starches were tested. Soluble potato starch (Reagent grade) was used as a reference. The other starches were obtained from A.E. Staley Manufacturing Company (P.O. Box 151, Decatur, Illinois 62525).

PFPS = Unmodified corn starch
ES-A = Eclipse A. lightly acid converted corn starch
ES-G = Eclipse G, highly acid converted corn starch
SD-5 = Stadex #5, lightly dextrinized corn starch
SD-80 = Stadex #80, highly dextrinized corn starch

Iodine Reaction and Carbon Determination

The starch iodine test is a widely used and simple test. However, as a quantitative test it is subject to a number of variables, specifically the molecular weight and bonding structure of different starches. Iodine reacts with amylase to yield a deep blue color complex with approximately one iodine molecule per 7-8 glucose units. (Physical Characteristics of Polysaccharide, Florkin and Stotz, p. 189). Iodine is believed to be complexed in the core of the helically arranged glucose chain. At least 30-35 glucose units are needed for full color development. Shorter chains give a red color with maximum absorption peaks in the 520 millimicron region. Chains of less than six glucose units give no color reaction.

Iodine reagent used for starch determination was made up periodically using 0.2 wt. % iodine in 2.0 wt. % solution of potassium iodide. Equal volumes of iodine reagent and sample were mixed and allowed to stand for 15 minutes. The color was measured in a Coleman Stectrophotometer at 520 and 680 millimicrons. Absorbance at 680 millimicrons is related to the concentration of amylase units with a long chain length of the order of 35 and higher. Absorbance at 520 millimicrons is related to the concentration of the shorter chain lengths, of the order of 8-12.

Carbon contents of starch solutions were measured in the Beckman Carbon Analyzer. All starches gave essentially equivalent results, and approach the theoretical value of 44.5 wt % carbon. However, there are substantial differences between starches in the iodine reaction as indicated below.

Starch	Concentration for 10% Transmittance, mg/l	
	520 m μ	680 m μ
Soluble starch	96.5	123
PFPS	160	148
ES-A	109	112
ES-G	102	114
SD-5	116	143
SD-80	93*	84**

* 50%
** 80%

Molecular Weights of Starches

Molecular weights were estimated from right angle light scattering measurements of concentrated solutions as described by Debye (Journ. Physical and Colloidal Chemistry 51, 18, 1947). Molecular weights are in the range 30 x 10^6 for the soluble starch to 220 x 10^6 for the ES-A sample after 5 minute autoclaving in distilled water. These values are substantially higher than the molecular weight determinations reported in the literature using chemical methods. Stacy and Foster (Journ. Polymer Science, 20, 57, 1956) have pointed out that physical methods of molecular weight measurement generally give higher values than the chemical methods; literature values based on chemical determinations are in the range of 160,000 to 360,000 grams per mole for corn amylo pectin whereas light scattering gave values in the range 40-100 x 10^6 grams per mole. One likely explanation for these differences is that starch is a mixture of different molecular weight fractions depending on the source as well as prior treatment. Thus it appears likely that processed starches would have a different mixture of high and low molecular weight constituents. The work of Stacy and Foster provides support for this explanation; they report a molecular weight of approximately 30 x 10^6 for limit dextrins prepared by B amylase degradation of corn amylo pectin as compared to 80 x 10^6 for the untreated amylo pectin. (It is assumed that B amylase does not catalyse internal hydrolytic scissions).

Jeanes (Encyclopedia of Polymer Science and Technology, Vol. 4.,) reports weight average molecular weights of 40-50 x 10^6 for dextrans produced in growing cultures of L. Mesenterioides. One dextran preparation showed a molecular weight of 97 x 10^6 (weight avarage) using light scattering and a number average molecular weight of 1.7 x 10^6 and 365,000 as determined by chlorous acid and copper reagent respectively.

Sephadex Gel Separation of Starches

Sephadex gel columns were used to evaluate the molecular size distribution of various starches. The objective was to determine the quantity of low molecular weight polysaccharides associated with each type of starch.

Three sizes of gel material were obtained from Pharmacia Fine Chemicals Inc. (800 Centennial Avenue, Piscataway, N.J.).

Sephadex Type	Molecular Weight Fractionation Range (Dextrans)
G-15	0-1500
G-75	1000-50,000
G-150	1000-150,000

The gels and the columns were prepared in accordance with the descriptive literature provided by the manufacturer. Columns 2.5 cm in diameter and gel beds of 60 cm. were used. The starch was layered into the column surface and eluted with distilled water. Eluent was tested for carbon (carbon analyzer) and starch using the iodine test. The results from the starch determination appear to be more sensitive. Typical eluent curves are shown in Figures 4 and 5. Blue dextran 2000 which has an approximate weight average molecular weight of 2×10^6 and is believed to have essentially no small polysaccharide fragments was used as a reference curve. The quantity of small diameter materials was calculated from the extension of the eluent curve for each starch.

Gel Column	G-15	G-75	G-150
	Percent of Starch	Retarded by	Gel %
Soluble Starch	0	0	0-19
PFPS	4	0-14	9
ES-A	0	5	8
ES-G	5	6	14
SD-5	2	18	32
SD-80	3	60	61

The results show that with the exception of SD-80 and SD-5 the starches have relatively small amounts of polysaccharide below 150,000 molecular weight, in the range of 0 - 14%. There is almost no material in the 0-1500 molecular weight range. The Stadex starches show substantial quantities of the lower molecular weight fragments; SD-5 has 32% below 150,000, molecular weight and 18% below 50,000 while the more dextrinized SD-80 sample has some 60% in the 1000 to 50,000 molecular weight range. Those observations are in line with the poor iodine color response of SD-5 and especially of the SD-80 sample. The SD series of starches appear to have been treated (dextrinized) to such a degree that molecular size does not allow for formation of the iodine complex.

FIGURE 4

SEPHADEX GEL SEPARATION OF SOLUBLE STARCH

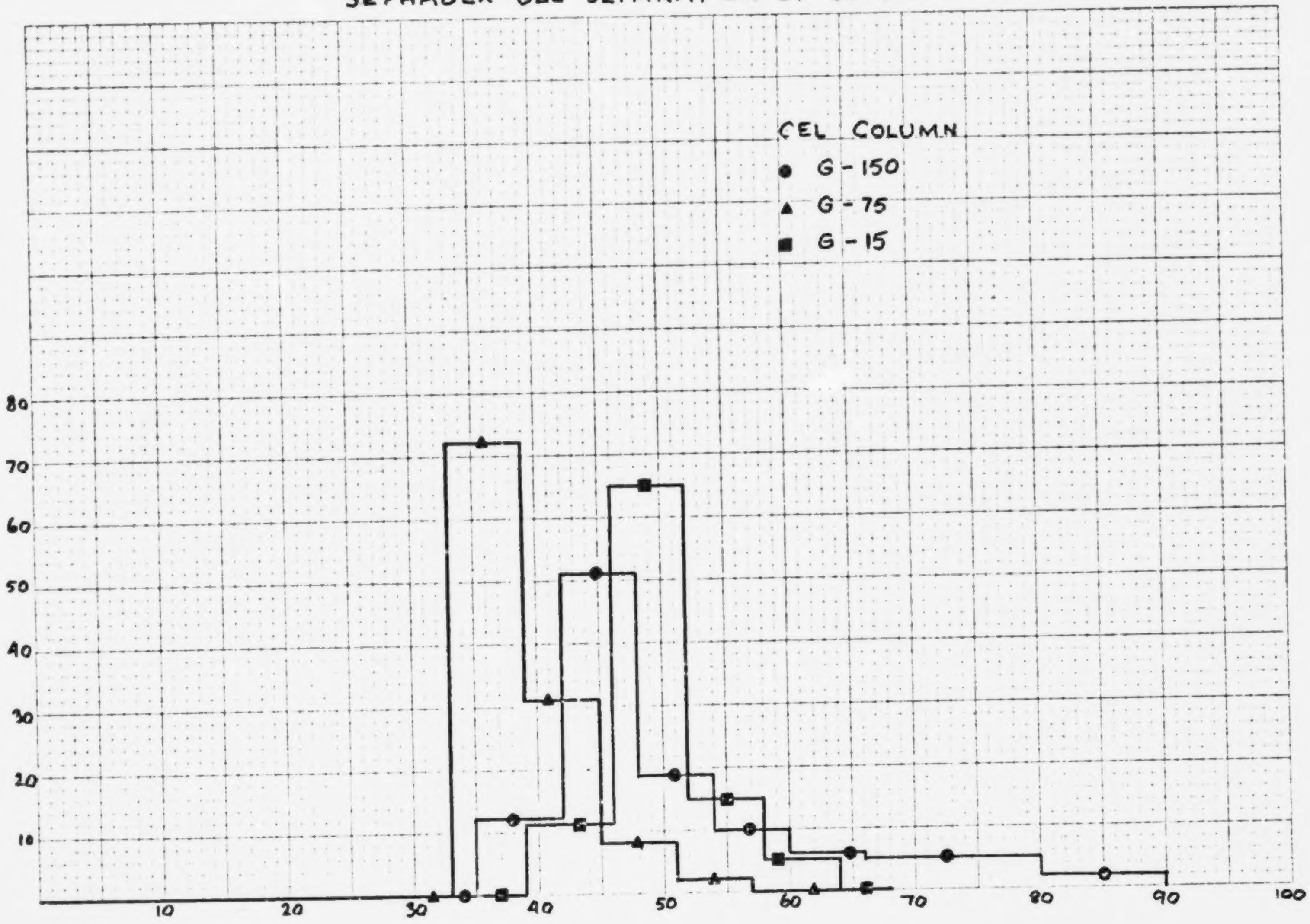
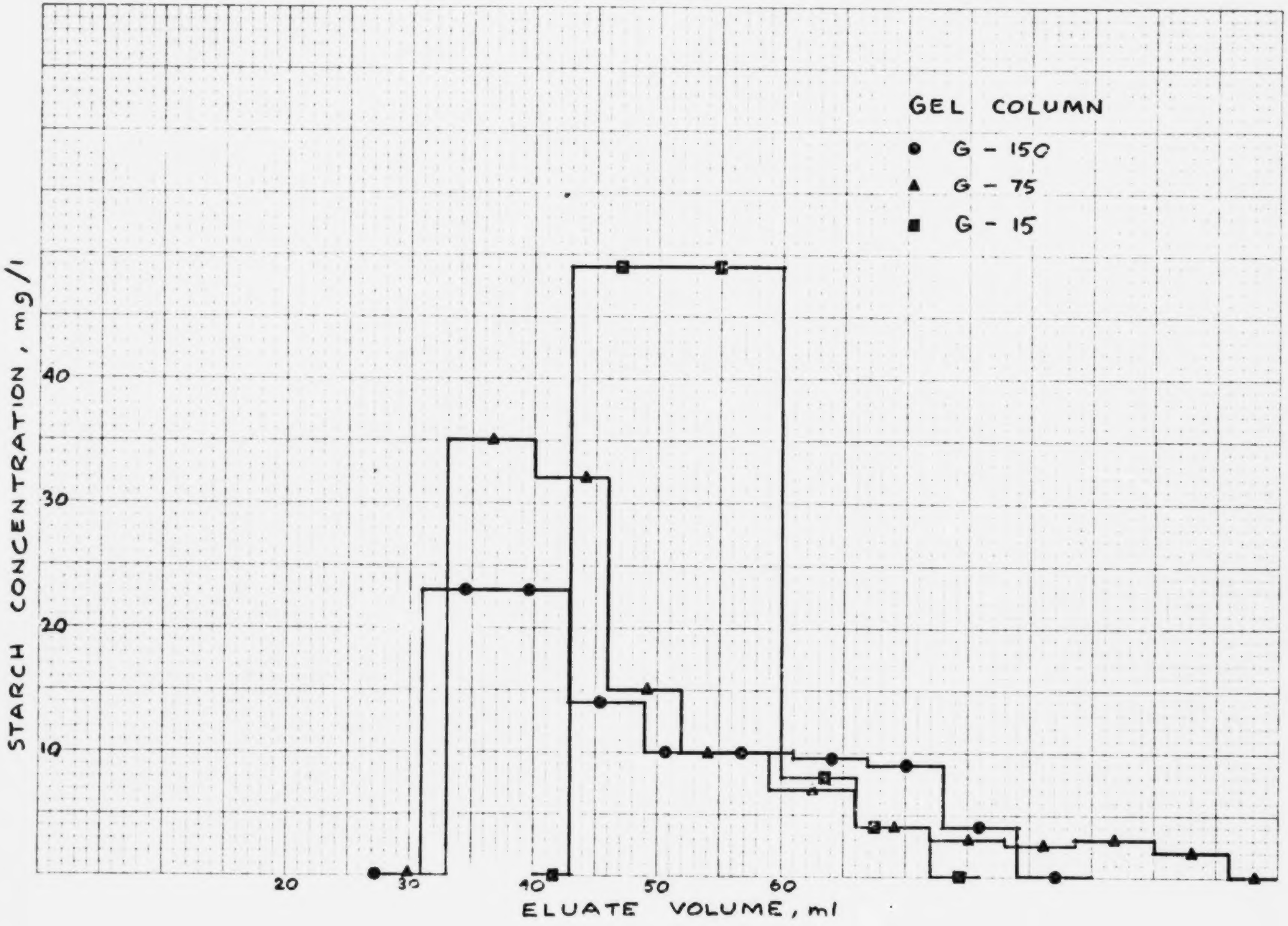


FIGURE 5

SEPHADEX GEL SEPARATION OF STARCH SD-5



Hydrolytic Degradation of Starch

Enzymatic hydrolysis of starch was measured in batch reactors using acclimated innoculum from a continuous propagator. Reactors were sparged with air to provide excess oxygen and to keep the system well mixed. The tests were designed to measure initial rates of hydrolysis at various substrate concentrations and to see how the rates of hydrolysis change with time.

Innoculum was collected overnight from a continuous propagator (described in section on Experimental Procedure). Soluble starch concentrate was prepared by autoclaving for 5 minutes. Small amounts of starch concentrate were added to aliquots of innoculum and starch concentrations were measured every 10 minutes using a modified iodine test as described earlier. After approximately one hour, another portion of starch concentrate was added and the starch concentration was again monitored.

Typical results are summarized in Figure 6 (complete data are listed in the appendix). Starch concentration drops off rapidly at the start of each test but slows down appreciably as the reaction proceeds. The rate of starch removal is highest in the test reactors with the highest initial concentration of starch. Rates of removal after the second addition of starch concentrate are not quite as high as the initial rates. Changes in rates are shown more explicitly in Figure 7. Rates of starch removal were calculated for each ten minute time increment, and compared to illustrate the effect of starch concentration and the effect of time. Figure 7 shows that the initial rate of removal is independent of starch concentrations above 100mg/l but drops off at lower concentrations.

The initial rate data appears to follow Michaelis-Menton enzyme kinetics which predict zero order reaction (in substrate) at high concentrations of substrate and first order reaction in substrate at low concentration. The calculated adsorption coefficient (Km) has a value of 35mg/l and the calculated (extrapolated) maximum rate of reaction (Vm) has a value of 66 mg/l per minute for the given enzyme concentration. The exact concentration of active enzyme is not known, however, suspended solids concentration and hence the biomass was measured by millipore filtration, (40.2, 38.9, 38.2, 37.7 mg/l for reactors A,B,C,D respectively). The calculated rate coefficient is therefore approximately 1.7 per minute (mg/l starch per mg/l biomass per minute).

The open points in Figure 7 represent measured rates of removal at subsequent time intervals. It is interesting to note the gradual decrease in rate of degradation with time, despite the continuing growth of new biomass which accumulates in the reactor, and is equivalent to an increase of 30 to 50% in biomass from the beginning to the end of the test. Furthermore, there was a noticeable increase in rate after the second addition of fresh starch substrate. One possible explanation for these

FIGURE 6

HYDROLYTIC DEGRADATION - EFFECT OF STARCH CONCENTRATION

EXPERIMENT # 4-2-68

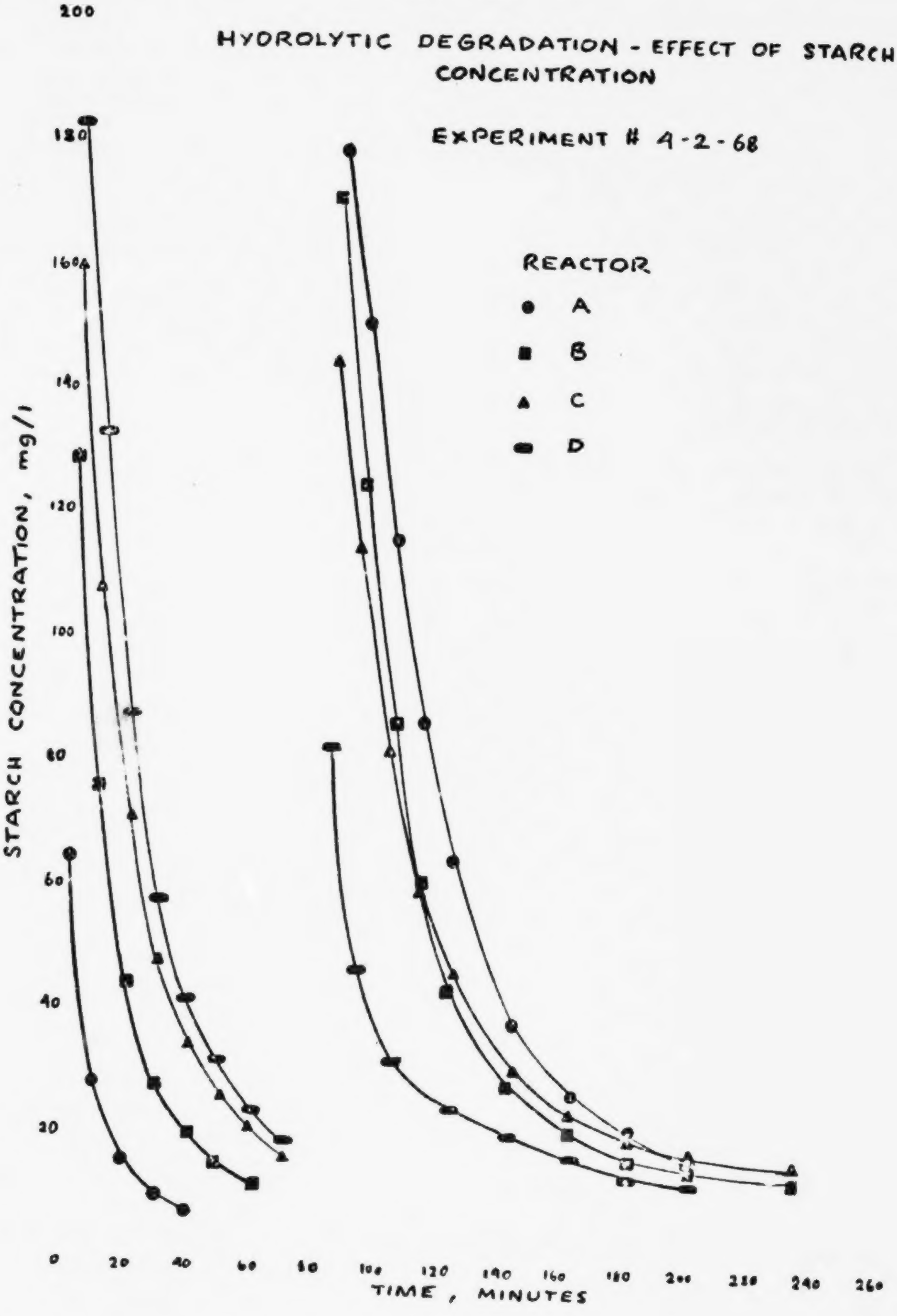
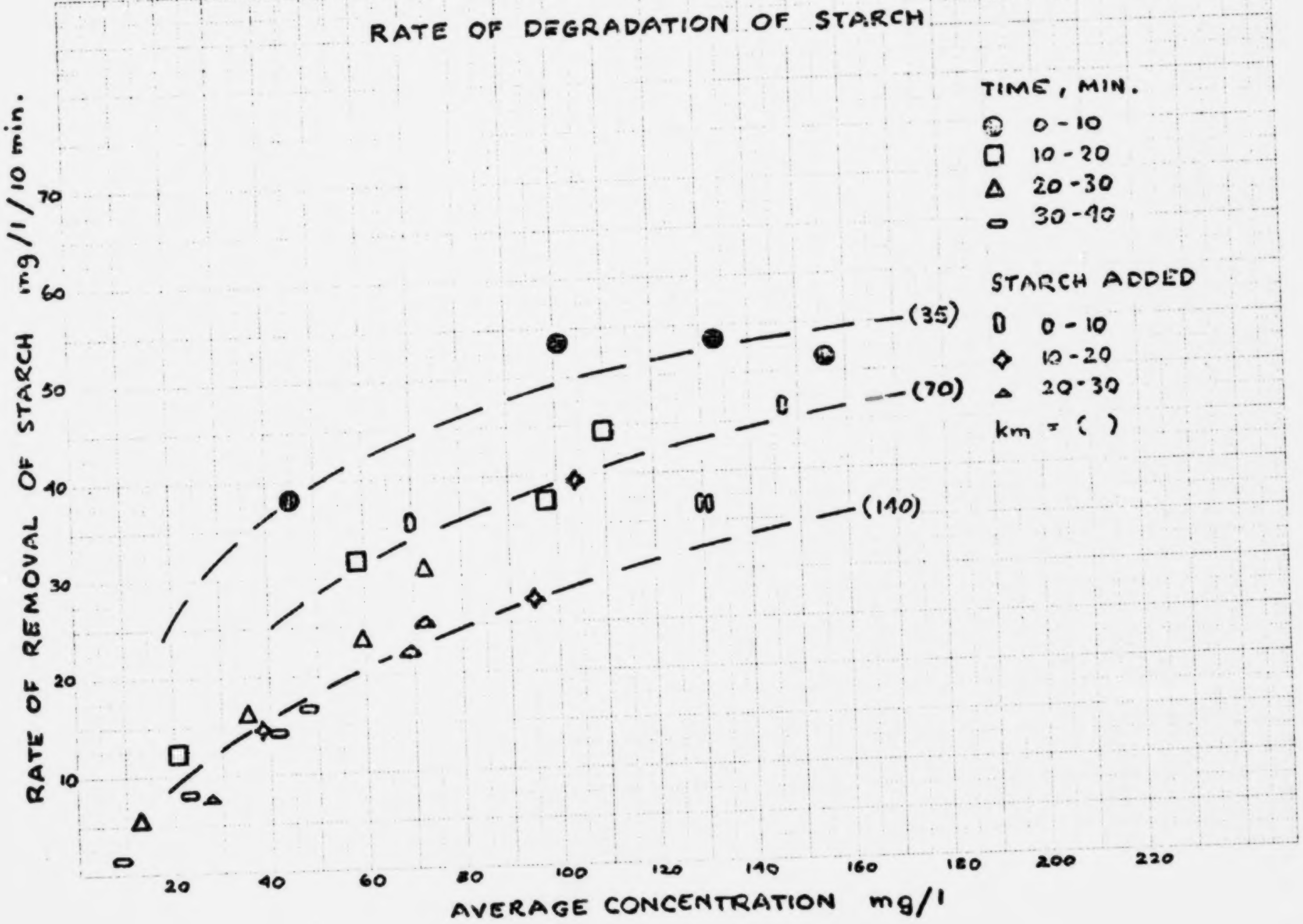


FIGURE 7



RATE OF DEGRADATION OF STARCH

observations is that hydrolyzed polysaccharide fragments act as competitive inhibitors of the hydrolytic enzyme; there should be a corresponding increase in the adsorption coefficient, (Km). The dashed lines in Figure 7 are calculated using progressively higher values of (Km) at otherwise constant conditions. Increasing (Km) by a factor of four accounts for most of the observed drop in rates.

Comparison of Biological Degradation of Different Starches

Rates of biological decomposition for each of the 5 starches were determined in batch tests. Innoculum was taken from a continuous propagator operating on soluble starch solution (100mg/l starch in salt solution). Starch concentrates (200mg/l) were prepared by autoclaving for 5 minutes to dissolve the starch in distilled water. (Details of procedure and data are listed in the Appendix under Exp. #4-30-68 and 5-7-68). Starch concentrate was added at the beginning of each test; air was sparged into each batch reactor to provide oxygen and to keep the system well mixed.

Starch concentrations are shown in Figures 8 and 9. More than 50% of the starch is degraded in the first 30 minutes; thereafter the rate of degradation slows down. The initial rates of degradation (first 30 minutes) are summarized and compared below.

Initial Rates of Starch Degradation

Starch	Exp. #	mg/l (first 30 minutes)	Relative Rate
Soluble starch	4-30-68	119	1.0
ES-A	4-30-68	100	.84
SD-5	4-30-68	103	.87
SD-80	4-30-68	78	.66
Soluble starch	5-7-68	134	1.0
PFPS	5-7-68	151	1.13
ES-6	5-7-68	115	.86

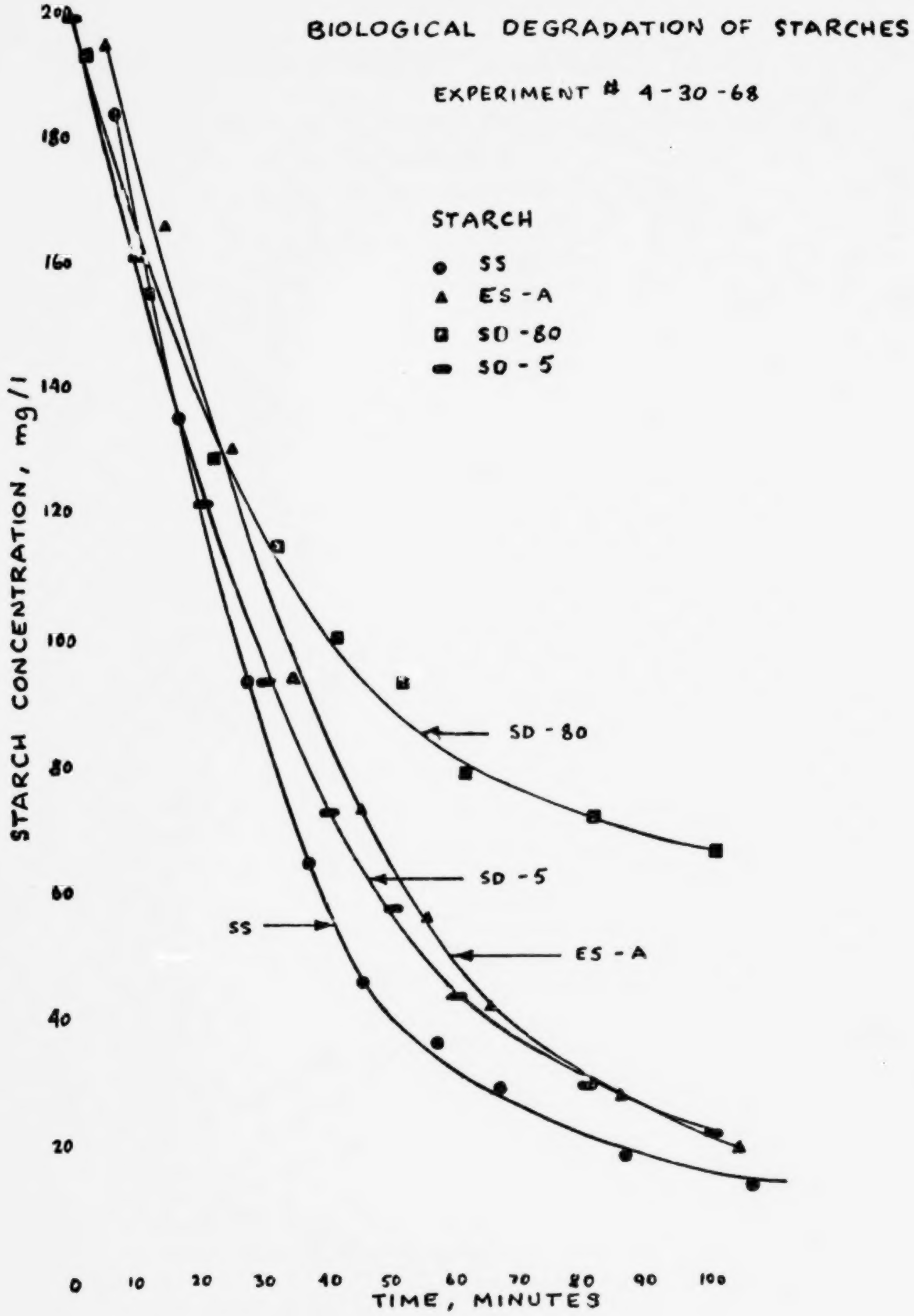
Unmodified corn starch (PFPS) shows the highest rate whereas the dextrinized starches show the lowest rates. The most highly dextrinized starch (SD-80) shows a tendancy to level off after 2/3 of the starch has been degraded, thus leaving a substantial fraction, about 1/3 that is apparently not degradable by the existing enzymes in the first few hours of the test.

A smaller number of millipore filtered samples were also tested for carbon content to determine the rate of removal of carbohydrates.

FIGURE 8

BIOLOGICAL DEGRADATION OF STARCHES

EXPERIMENT # 4-30-68



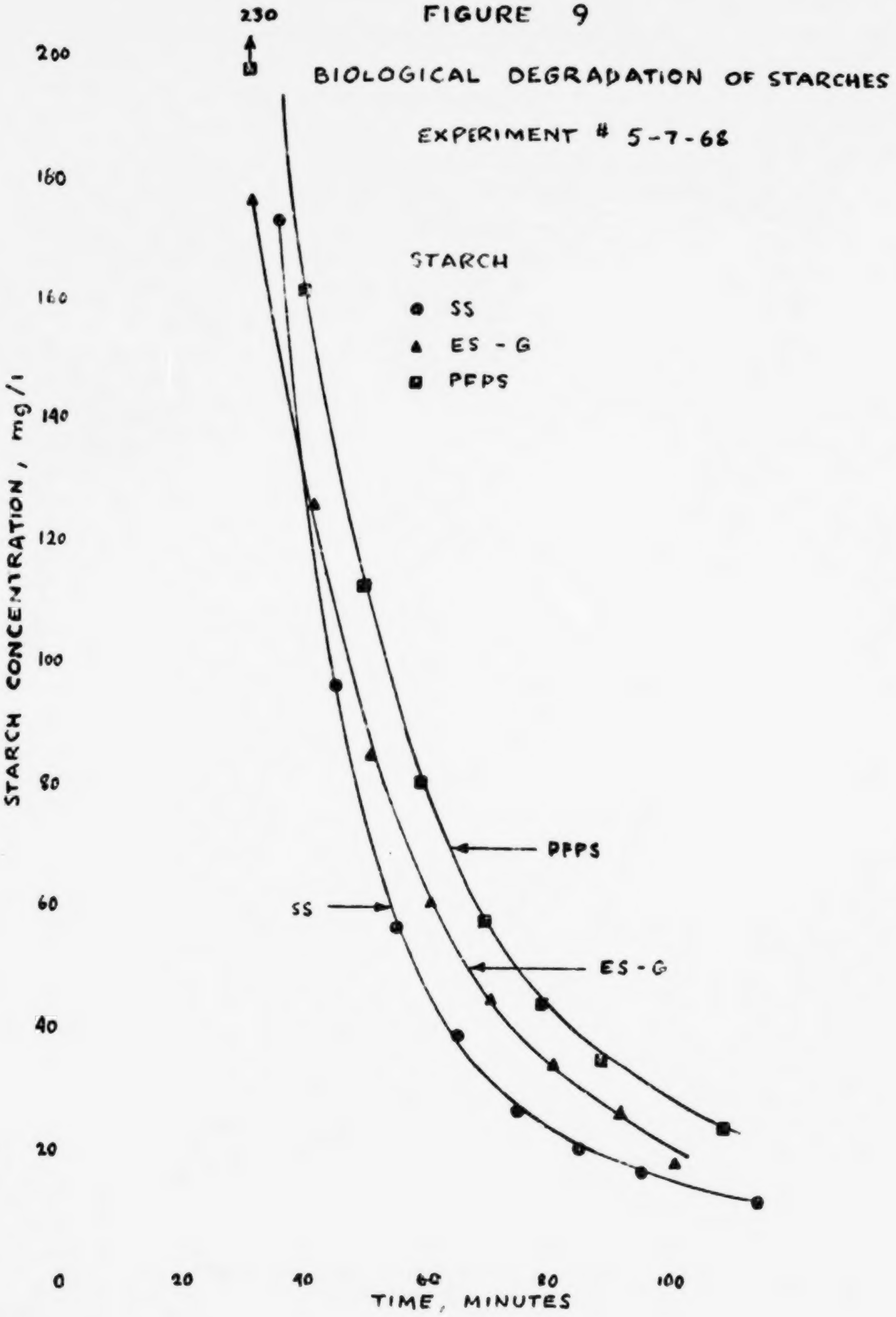


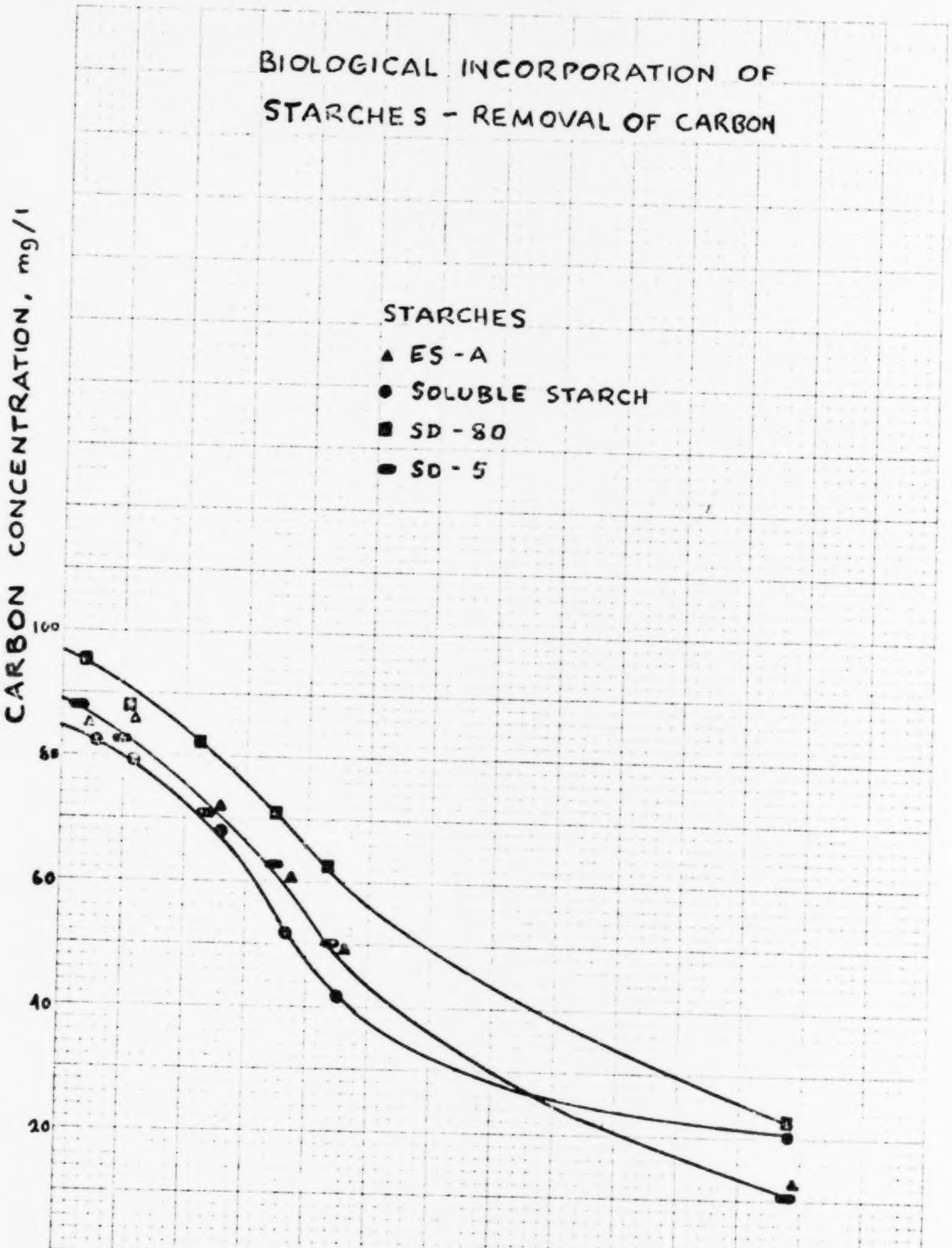
FIGURE 9

BIOLOGICAL DEGRADATION OF STARCHES

EXPERIMENT # 5-7-68

Disappearance of carbon was taken to represent biological growth because millipore filtration removes bacterial solids but does not remove the starch polysaccharide products of hydrolysis. Figure 10 summarizes the data and shows that carbon removal pattern is essentially similar for all starches. The sigmoidal curve is typical for this type of batch test. The rate of carbon removal increases for the first few hours while the biomass undergoes exponential growth; as growth proceeds substrate becomes limiting thereby reducing growth rate; the rate of carbon removal also slows down and approaches zero (constant concentration). The residual carbon concentration of 10-20 mg/l is quite typical for batch tests and probably represents non-biodegradable carbon for biomass that is available. The implications of this residual carbon are discussed elsewhere in this report. Close inspection of the data on carbon removal shows that the rate of carbon removal for starch SD-80 is somewhat slower than that for the other starches. The effect is most likely associated with the slower rate of hydrolysis of the SD-80 starch which in turn slows down bacterial incorporation of hydrolyzed carbohydrates. The precise magnitude of the difference in rate of carbon removal has not been established but could be as much as 50% in the course of the test.

FIGURE 10



BIOLOGICAL INCORPORATION OF STARCHES - REMOVAL OF CARBON

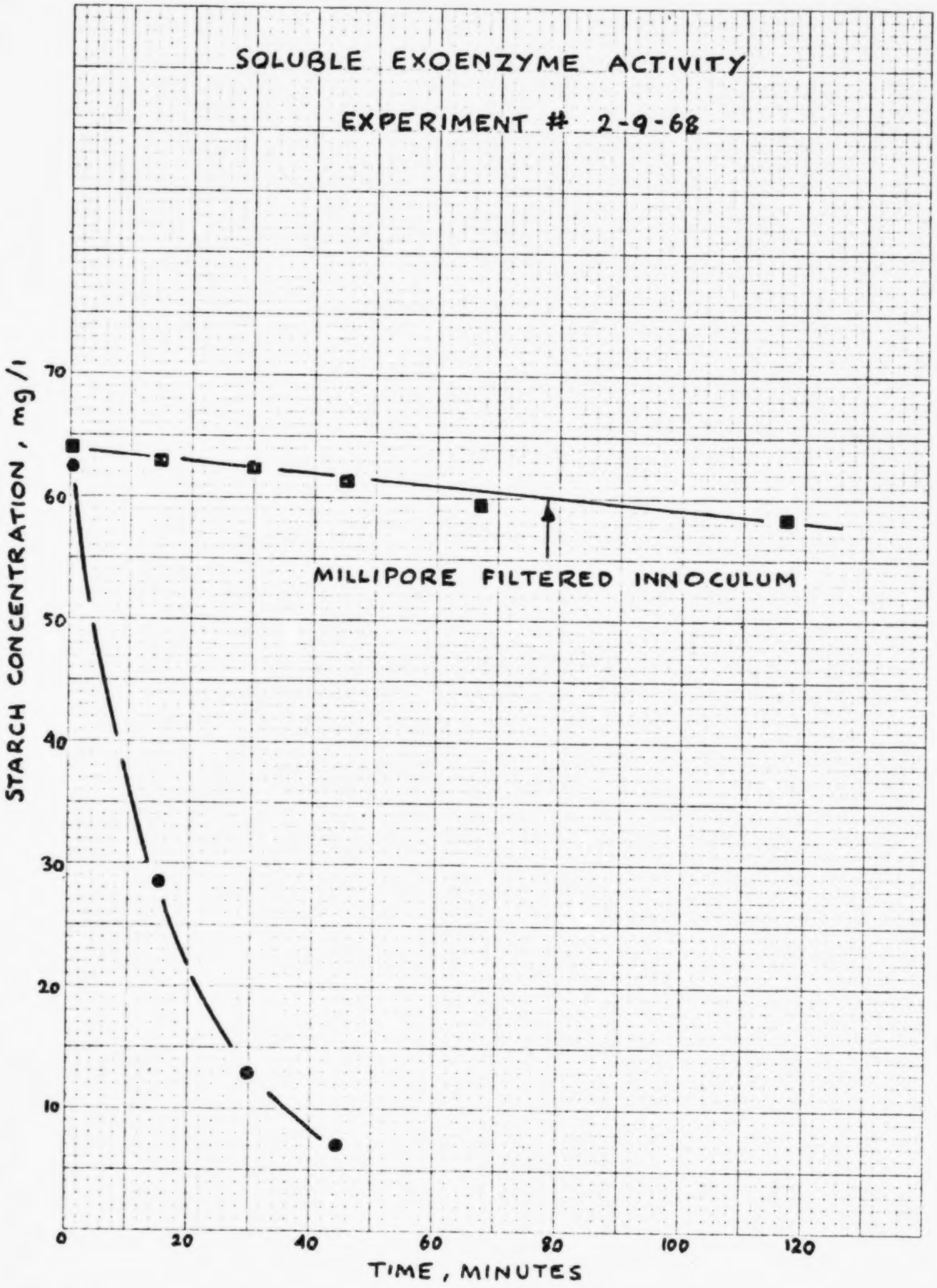
Soluble Exoenzyme Activity

The location and control mechanisms of the hydrolytic enzymes that catalyze starch degradation are not well defined. There is considerable support for the concept that in bacterial systems exo-enzymes are closely associated with cell walls. Some enzymes are also released to the medium in the form of small colloids which pass through 0.45 micron filters and will be referred to as "soluble" enzymes. The following experiments were designed to test for the presence of soluble enzymes in bacterial decomposition of starches.

Innoculum was obtained from a continuous propagator using soluble starch feed solution (100mg/1). Fresh feed solution was added at the beginning of each test; one reactor received the innoculum as collected, the other reactor received millipore filtered innoculum (0.45 micron). Starch and carbon concentrations were measured for a period of several hours; results are summarized in Figure 11 detailed data are listed in the Appendix as Experiments #2-9-68 and 2-10-68. There is evidence of exoenzyme activity, however, the observed rate is only 3% of innoculum. Similar results have been reported by other investigators. Banerji (University of Illinois, Sanitary Engineering Report, No. 29) has demonstrated the presence of hydrolytic enzymes in activated sludge liquors. Millipore filtration of mixed liquor (0.45 micron filter) showed substantial amylase activity; cell free filtrates from acclimated sludges in the endogenous phase showed higher activity than filtrates from sludges in or near the log phase of growth, 150mg/1/hr versus 20mg/1/hr respectively. Thus, it appears that prior history and age of the biomass is an important variable controlling production and release of exoenzymes.

This idea was confirmed in a test of effluent collected form the inclined plane test reactor. Filtered effluent showed about 18% of the hydrolytic activity observed on unfiltered effluent. The slime layer consists of considerably older biomass and some of which is undergoing endogenous metabolism and releasing "soluble" enzymes to the feed solution passing over the slime layer.

FIGURE 11


SOLUBLE EXOENZYME ACTIVITY
EXPERIMENT # 2-9-68
MILLIPORE FILTERED INNOCULUM
STARCH CONCENTRATION, mg/l
70
60
50
40
30
20
10
0
20
40
60
80
100
120
TIME, MINUTES

Degradation of Starch-Glucose Mixtures.

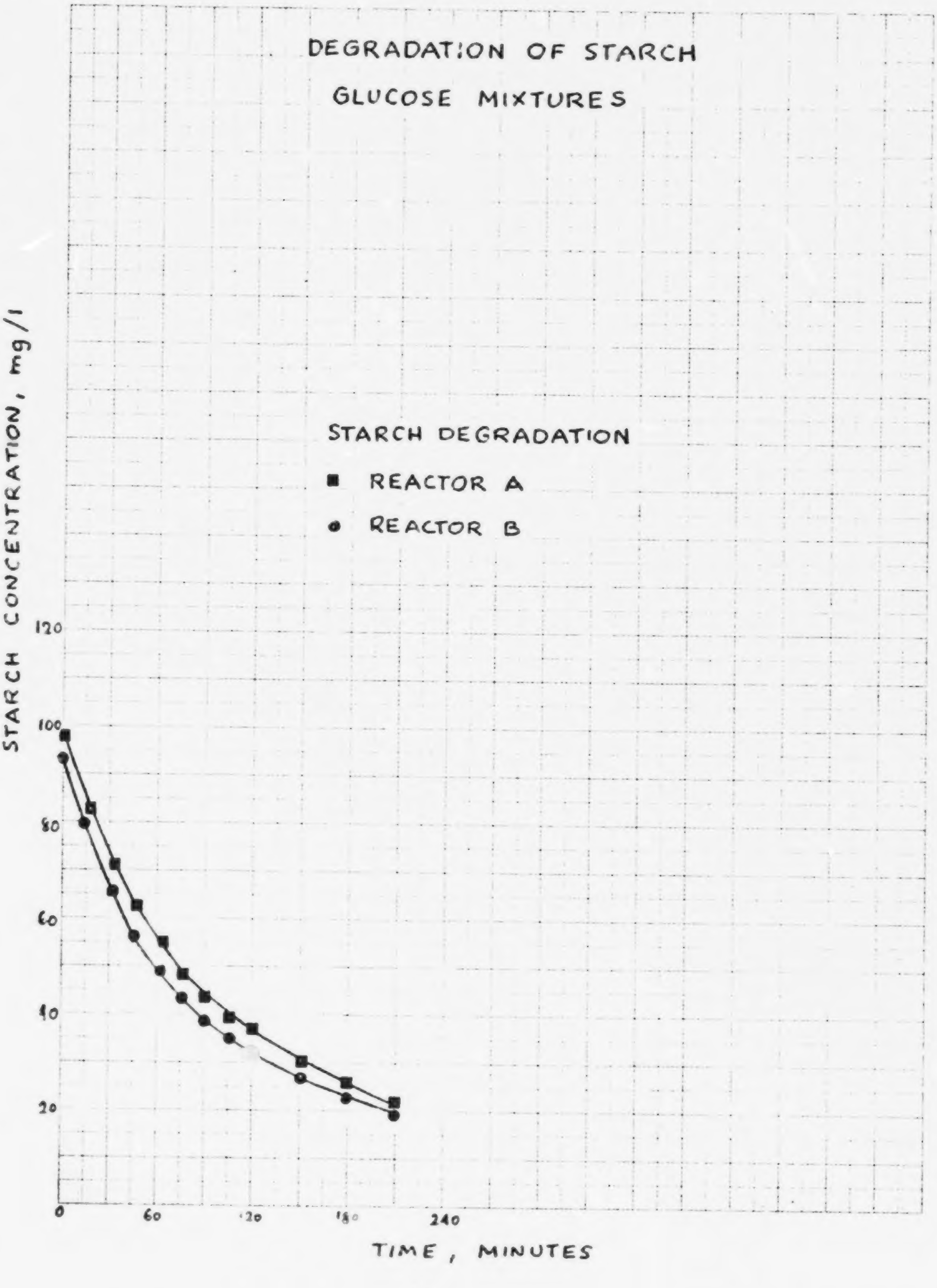
A series of batch tests were carried out to measure the effect of adding glucose and starch substrate to an innoculum that was previously acclimated to starch as the only source of carbon. Concentrated solutions of glucose and starch were added at the start of each test;

Reactor	Nominal Initial Concentrations, mg/l	
	Glucose	Starch
A	0	100
B	100	100
C	100	200
D	100	0

See appendix Table Experiment No. 8-29-68 and 9-4-68 for detailed description. Samples were removed periodically and tested for glucose, carbon, and starch concentration.

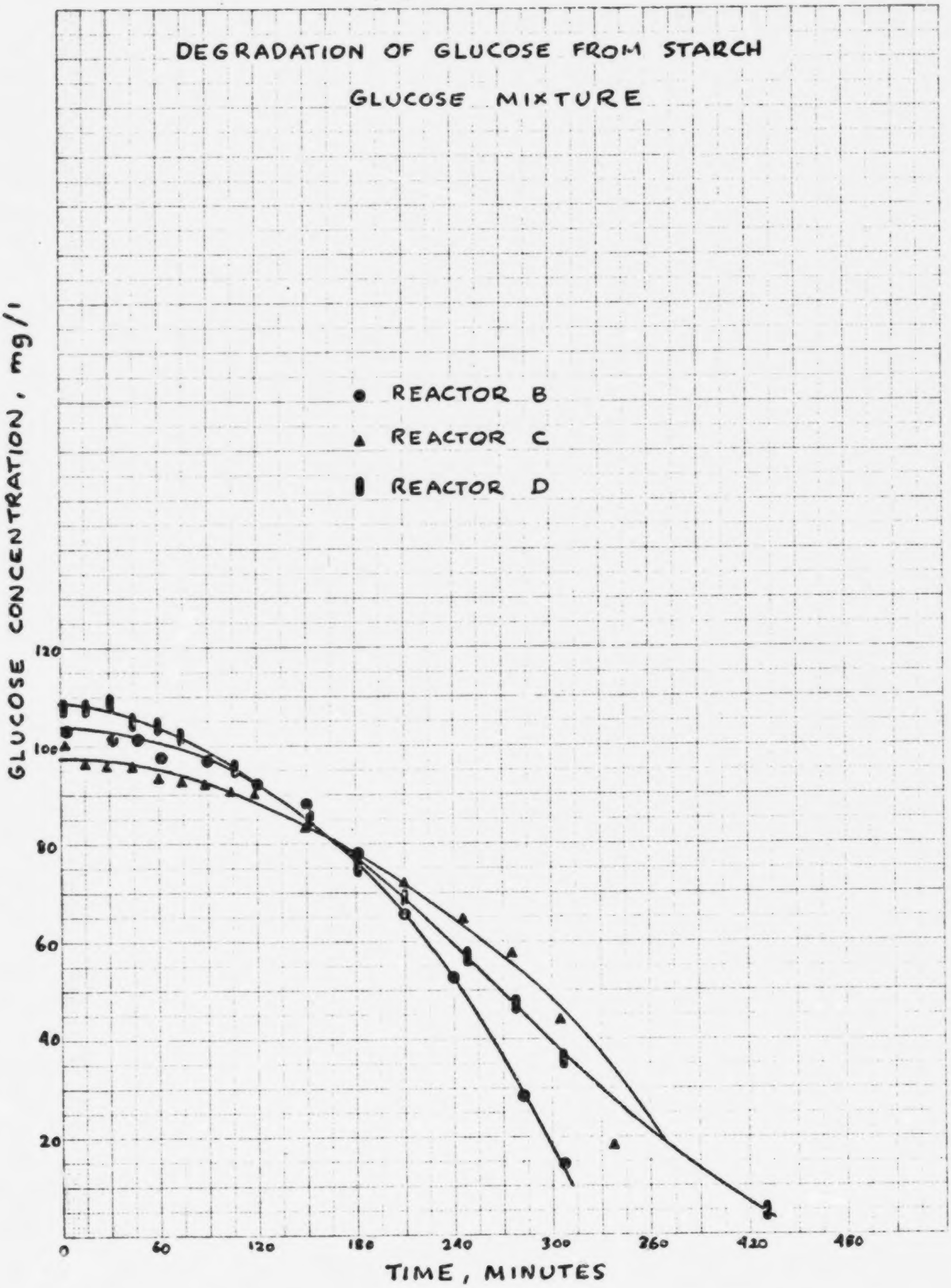
Introduction of glucose with the feed solution has no measurable effect on the hydrolytic degradation of starch as illustrated in Figure 12. The decay curve for starch is typical and shows a high initial rate which then levels off at lower starch concentrations. Comparisons of the rates of glucose degradation are shown in Figure 13. The presence of starch appears to slow down the initial rate of glucose utilization. However, the effect is small at equal concentrations (100mg/l each) and some 20% greater when 200mg/l of starch was added along with 100mg/l of glucose. The rate of disappearance of carbon is essentially the same in all tests, indicating that growth of biomass is not affected by the relative concentration of glucose and starch when the innoculum is acclimated to starch feed. However, the accumulation of biomass is greater at the end of the tests which received the largest amount of degradable carbon.

FIGURE 12



DEGRADATION OF STARCH GLUCOSE MIXTURES

FIGURE 13


DEGRADATION OF GLUCOSE FROM STARCH
GLUCOSE MIXTURE
● REACTOR B
▲ REACTOR C
▮ REACTOR D
GLUCOSE CONCENTRATION, mg/l
120
100
80
60
40
20
0
60
120
180
240
300
360
420
480
TIME, MINUTES

Amino Acid Metabolism

Microbial utilization of amino acids has received considerable attention in the Microbiology, Biochemistry and Sanitary Engineering Literature. Rates and mechanisms of catabolism, and rates of direct incorporation into protein have been described. Amino acid transport has been studied for a number of microorganisms. Kay and Gronlund (Journ. Bact., 97, p. 273; 1968) have presented an interesting comparison of the rate of transport for *Pseudomonas aeruginosa.* The results show large differences between amino acids, 40 fold differences from a low of 1.92×10^{-4} micro-moles per minute per milligram by weight of cells to 80×10^{-4} μ moles/min. - mg. (dry weight) as shown below.

Amino Acid	Rate of Transport*
Hydroxyproline	0
Cysteine	1.92×10^{-4}
Threonine	3.54×10^{-4}
Aspartate	4.11×10^{-4}
Serine	4.20×10^{-4}
Glutamic	4.67×10^{-4}
Proline	5.75×10^{-4}
Glycine	5.90×10^{-4}
Tyrosine]	1.40×10^{-3}
Methionine	1.99×10^{-3}
Valine	2.36×10^{-3}
Histidine	2.74×10^{-3}
Alanine	2.76×10^{-3}
Phenylalanine	3.23×10^{-3}
Lysine	3.36×10^{-3}
Isoleucine	3.92×10^{-3}
Tryptophane	5.20×10^{-3}
Arginine	6.75×10^{-3}
Leucine	8.00×10^{-3}

*Micromoles/minute - mg. of dry weight of cells using an external concentration of 10^{-6} moles/liter.

These values are particularly interesting because they also provide some insight on the relative rates of removal of amino acid from the surrounding medium; removal rates are in the range of .02 to 0.1 wt. % on cell mass per minute; thus indicating that these cells are highly effective for the removal of amino acids from dilute solutions. It has also been shown that incorporation of amino acids is energy dependent, and is probably mediated by transferases, therefore energy metabolism is a prerequisite for incorporation of amino acids. The transfer mechanism appears to be extremely effective; during exponential growth observed amino acid concentrations in the cell interior are 100 to 300 fold greater than in the medium.

Cells which had been in the stationary growth phase for 3 hours, showed essentially complete removal of amino acids, except for methionine, from the surrounding medium and from the cell interior.

Based on their results and extensive review of the literature, Kay and Gronlund conclude that:

> "The enzymatic nature of metabolite incorporation into cells, first demonstrated with carbohydrates, has been extended in recent years to encompass the amino acids, as well as other compounds. The data presented here revealed that the amino acid transport systems of *P. aeruginosa* have all the properties ascribed to active transport systems; the transport process is seemingly energy dependent, temperature sensitive, and saturates at high substrate concentrations.
>
> Amino Acids which are transported into *P. aeruginosa* equilibrate with amino acids synthesized de novo which reside in the intracellular pool, and also with amino acid pools which have been transiently preestablished from an exogenous source. This demonstrates that, in this organism, as in *E. coli* and in *Neurospora crassa*, incorporated amino acids equilibrate with, and are subject to, the same metabolic fates as amino acids synthesized de novo.
>
> The total amino acid pool levels for *P. aeruginosa* during exponential growth are of the same order of magnitude as the levels reported for *E. coli*."

It has also been demonstrated that *P. aeruginosa* can utilize the majority of commonly occurring amino acids for growth as either the sole carbon source or as the sole source of nitrogen (*Journ. Bact.* *100*,276-282, 1969).

Utilization of amino acids by activated sludge has been studies by several investigators. Carlson and Polkowski (*Journ. Water Pol. Control Fed.*, *34*, 816, 1962) showed that activated sludges can be maintained on single amino substrates as the nitrogen source. Acclimation of the sludge before exposure to a single amino acid increased rates of metabolism, most noticeable with glycine and to a lesser extent with

L-cystine and L-arginine. Rate of glycine depletion was reported to be 48 mg glycine per hour per gram of sludge solids. This is equivalent to 0.08 wt. % on cell mass per minute and is in reasonably close agreement with the values reported in the microbiology literature for incorporation rates. Malaney and Gerhold (*Journ. Water Pol. Cont. Fed.*, *41,* R18, 1969) list a number of studies evaluating the rates of utilization of amino acids under aerobic conditions. The individual amino acids showed substantial variations in oxidizability as measured by oxygen uptake. However, their conclusion is that amino acids can be considered as a family of similar compounds readily susceptible to biological oxidation. The reported oxygen uptake is reproduced below:

Compound	Percentage of Theoretical Oxygen Demand		
	6hr.	12hr.	24hr.
Aminoethanoic acid (Glycine)	4.1	8.1	16.9
2-aminopropanoic acid (α-DL-alanine)	11.7	27.0	43.0
3-aminopropanoic acid (β-alanine)	1.7	6.9	16.0
2-amino-3-methylbutanoic acid (DL-valine)	2.1	4.3	9.4
2-amino-3-hydroxypropanoic acid (L-serine)	8.6	21.0	29.0
2-amino-3-mercaptopropanoic acid (cysteine)	7.5	8.6	11.2
Dicysteine (L-cystine)	1.5	2.4	4.7
2-amino-3-hydroxybutanoic acid (DL-threonine)	3.9	8.2	16.2
2-amino-4-hydroxybutanoic acid (DL-homoserine)	1.1	2.3	4.4
2-amino-4- methylthiobutanoic acid (DL-methionine)	1.5	2.4	2.6
2-aminohexanoic acid (DL-norleucine)	2.3	5.2	12.9
2-amino-3-methylpentanoic acid (L-isoleucine)	2.4	5.3	14.8
2-amino-4-methylpentanoic acid (L-leucine)	1.4	3.6	9.9
2-aminobutanedioic acid (DL-aspartic acid)	8.9	16.2	28.8
2-aminopentanedioic acid (L-glutamic acid)	14.0	25.1	35.8
2-aminobutanedioic amide (L-asparagine)	10.3	19.5	24.7
2-aminopentanedioic amide (L-glutamine)	9.2	19.2	30.7
2,6-diaminohexanoic acid (L-lysine)	1.8	4.5	14.1
2-amino-5-quanidopentanoic acid (L-arginine)	2.1	7.7	16.5
2-amino-3-phenylpropanoic acid (DL-phenylalanine)	2.2	5.6	16.4
2-amino-(4-hydroyphenyl) propanoic acid (DL-tyrosine)	3.4	9.7	22.6
1-pyrrolylmethanoic acid (L-proline)	4.8	13.1	25.5
1-(2-hydroxy) pyrrolylmethanoic acid (L-hydroxyproline)	1.0	2.9	18.2
2-amino-3-imidazolypropanoic acid (L-histidine)	2.8	6.3	16.5

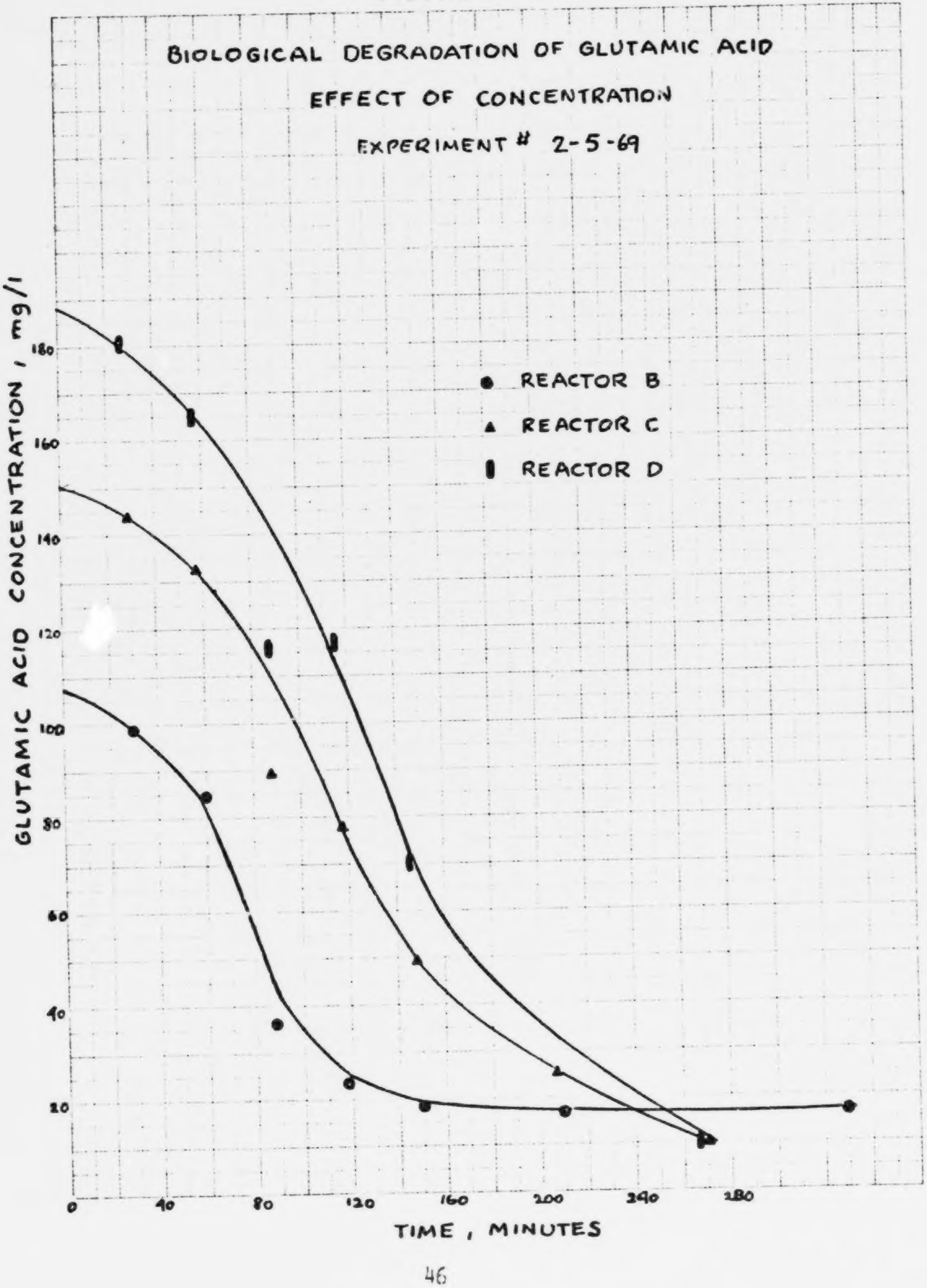
2-amino-3-indolypropanoic acid (DL-tryptophane)	0.6	1.4	4.6
N-acetyl-2-aminoethanoic acid (acetylglycine)	9.3	10.0	18.5
Glutamylcysteinylglycine (glutathione)	5.1	8.2	22.0

The theoretical oxygen demand represents a calculated value based on complete combustion to CO_2 and H_2O. The low oxygen demand for glycine as opposed to glutamate is consistant with the notion that amino acids are utilized for energy, as a source of nitrogen and also incorporated into cellular protein directly. The small carbon chain of glycine is obviously not as readily incorporated into the metabolic pathways as the larger glutamic acid carbon chain.

Glutamic Acid Utilization

A series of batch tests were carried out to measure rates of biological degradation of glutamic acid at various feed concentrations. Innoculum was obtained from a continuous propagator using 100mg/l glutamic acid feed solution without any other sources of either carbon or nitrogen. Concentrated glutamic acid solution was added to each reactor at the beginning of each test and samples were withdrawn periodically. The ninhydrin test was used to measure glutamic acid concentrations and carbon concentrations were measured independently with the Beckman Carbon Analyzer. Typical decay curves of glutamic acid as measured by ninhydrin are shown in Figure 14. The results show an increasing rate of removal initially, followed by a decreasing rate as substrate concentration drops off. The initial rates are essentially independent of substrate concentration. Data for carbon concentration follow a similar trend. Analysis of the data in terms of the simplified rate equation for batch systems as described earlier confirms the conclusion that utilization of glutamate is independent of concentration except at very low concentrations. Figure 15 shows that the rate data for carbon removal for all 3 reactors fall on a single straight line regardless of initial substrate concentration. The Monod adsorption coefficient (Kc) has been estimated to be approximately 16mg/l. This estimate is probably on the high side because it is based on residual organic carbon concentration which includes the concentration of nonbiodegradable carbon residues. The corresponding growth rate coefficient is 0.0073 per minute and the yield coefficient was measured to be 0.33 grams cell mass per gram of glutamic acid utilized. The yield coefficient is consistent with values reported in the literature.

FIGURE 14



BIOLOGICAL DEGRADATION OF GLUTAMIC ACID

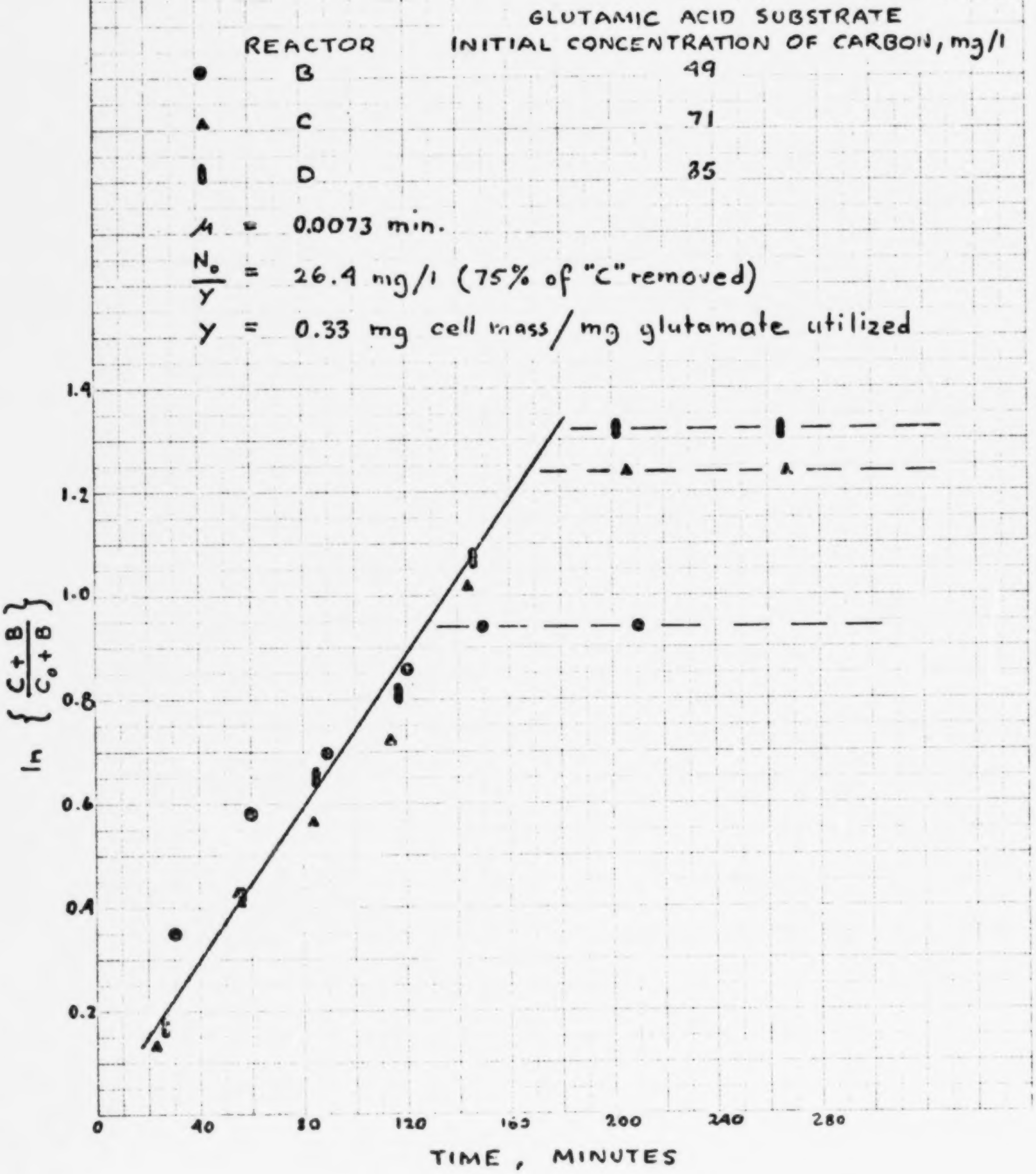
EFFECT OF CONCENTRATION

EXPERIMENT # 2-5-69

FIGURE 15

BIOLOGICAL DEGRADATION OF GLUTAMIC ACID

RATE OF CARBON REMOVAL - EFFECT OF CONCENTRATION



Glycine Utilization

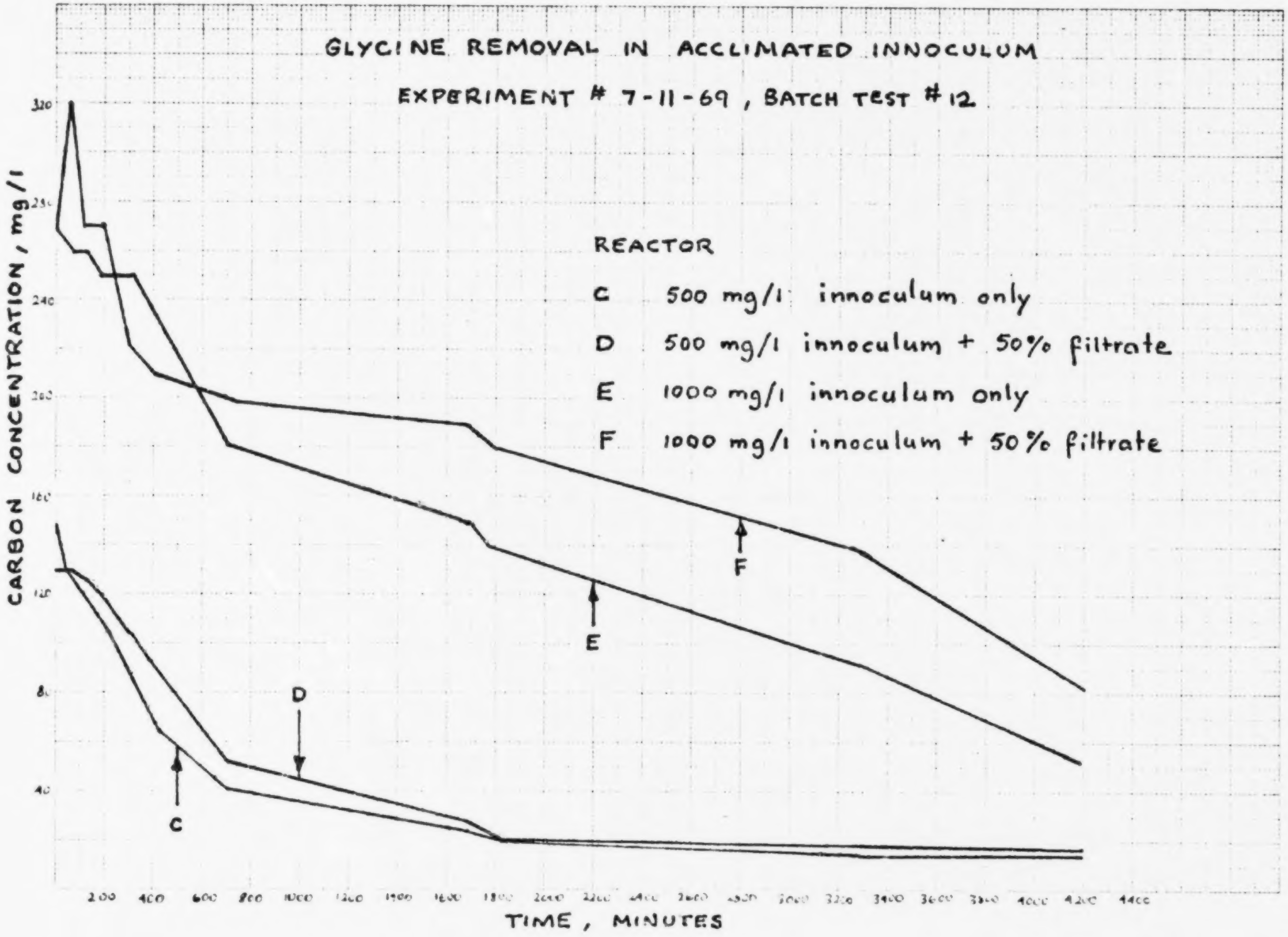
Glycine was evaluated in a series of tests to determine the effect of substrate concentration and the effect of acclimation of the innoculum. Experiment No. 2-20-69 was carried out using innoculum from a continuous propagator, using glutamic acid as the only source of carbon. The results show essentially no removal for the first 6-12 hours. It is particularly interesting to note that longer incubation periods were needed when higher initial concentrations of glycine were used. Carbon removal showed similar trends. Residual carbon at the end of the test (after 24 hours) accounted for 26-32% of the original feed carbon from glycine. Rate of glycine utilization was determined using an acclimated innoculum obtained from 3 continuous propagators using glycine feeds of different concentrations, 100, 500 and 1000 mg/l respectively. (Experiment No. 7-11-69). Half the effluent from each propagator was millipore filtered to remove cell mass and the filtrate added back. This allowed making comparisons of the effect of cell mass concentration as well as substrate concentration. The results show that the growth rate coefficient (μ max) is in the range of 0.0020-0.0049 and the Monod adsorption coefficient is approximately 80mg/l. The growth rate coefficient for the acclimated innoculum is comparable to the values observed for glutamic acid. However, the high value of the adsorption coefficient is unusual. The most likely explanation for this phenomenon is the presence of protozoa which act as predators and retard bacterial growth accumulation. In effect, substrate removal kinetics become linear after a short initial period of exponential growth. The subsequent prey-predator balance results in a uniform rate of glycine removal as shown in Figure 16.

As a further check on glycine utilization a new series of batch tests was carried out using glycine acclimated innoculum. Glycine and glutamic acid concentrates were added in different proportions as shown in Appendix Table 15 (Exp. No. 9-22-69). The carbon data are summarized in Figure 17. Glycine carbon is not utilized as rapidly as glutamic acid as shown by the slower rate of carbon removal in Reactors A and B. The highest rate of removal is observed in reactor C and E where glutamic acid concentration is 50mg/l. These data confirm earlier indications that glycine is not as readily utilized as the larger molecular weight amino acids.

Protein Chemistry

Proteins are polymers of amino acids which are linked by peptide bonds between the carboxyl and amino groups of adjacent amino acids. Elemental compositiontells relatively little of the structure or function but approximates 45-55 wt. % carbon, 6-8 wt. % hydrogen, 19-25 wt. % oxygen and 14-20 wt. % nitrogen. Sulfur and phosphorous are also present in small amounts. Molecular weights range from 10,000 to 10^6, which still places proteins in the collodial size range which passes through 0.45 micron millipore filters.

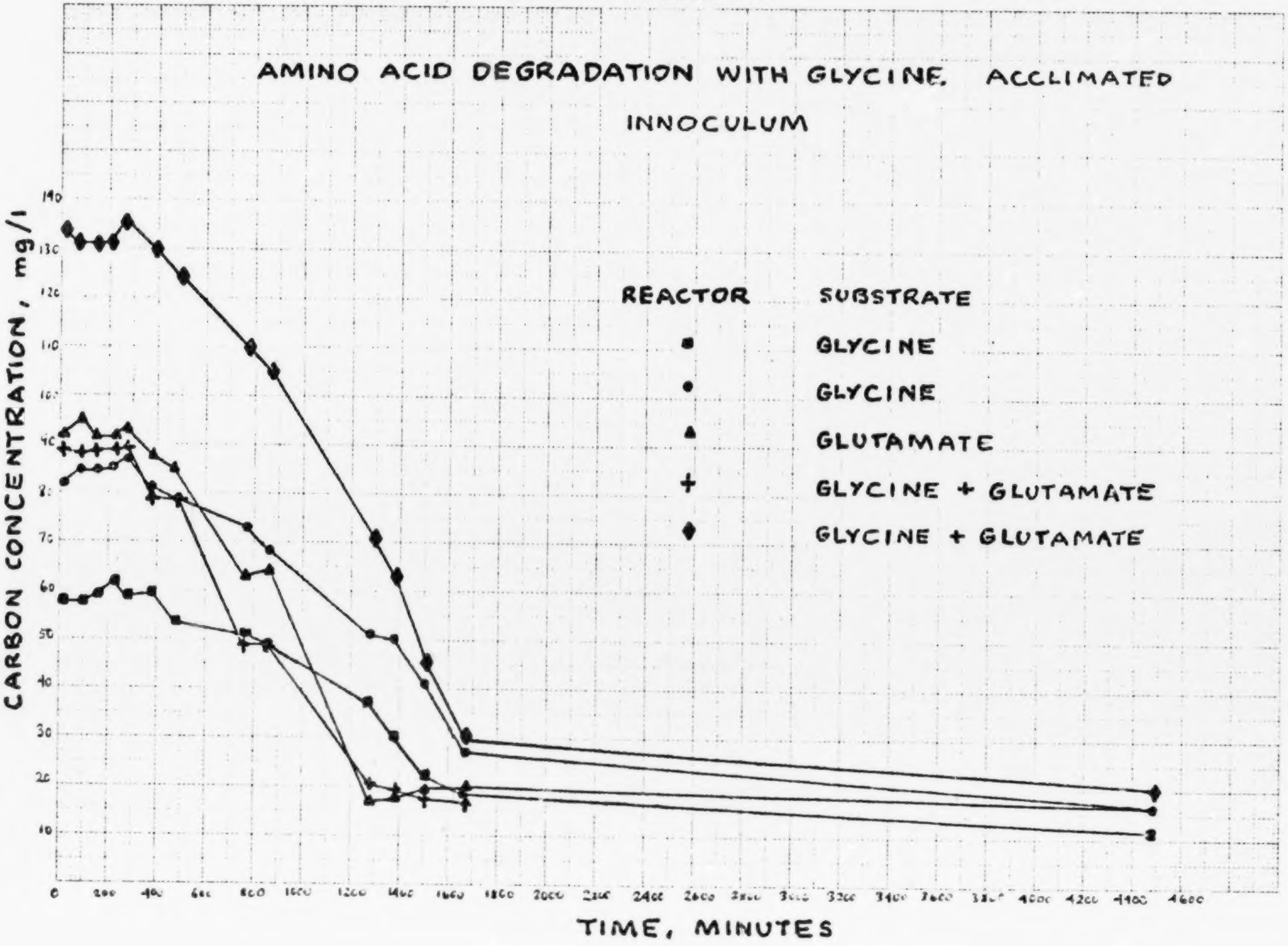
FIGURE 16



GLYCINE REMOVAL IN ACCLIMATED INNOCULUM

EXPERIMENT # 7-11-69, BATCH TEST # 12

FIGURE 17



AMINO ACID DEGRADATION WITH GLYCINE. ACCLIMATED INNOCULUM

The protein used in this work was obtained from Difco Laboratories (Detroit, Michigan) under the trade name Bacto Peptone. Typical composition is listed below:

	Wt. %
Total Nitrogen	16.16
Peptone Nitrogen	15.38
Ammonia Nitrogen	0.04
Organic Sulfur	0.33
Inorganic Sulfur	0.29
Phosphorous	0.22
Ash	3.53

Hydrolysis of proteins yields amino acids and polypeptide intermediates. Hydrolysis is mediated by acids or alkalies in boiling water or by certain proteolytic enzymes. Enzymatic hydrolysis yields free amino acids.

An excellent review of the proteolitic enzymes and hydrolysis of peptide bonds is presented by Boyer (*The Enzymes Vol. III,* Academic Press 1971, and *Vol. IV,* 1960, p. 193). There has been a considerable accumulation of information on the mechanism, rates and products of protein hydrolysis. Matsubara (as above, p. 721) points out that microbial proteases are predominently extracellular and can be isolated from filtrates of bacteria, molds and yeasts. Most of the common proteolytic enzymes of animal origin are present in the filtrate from bacterial and mold or yeast cultures, including amino- and carboxypeptidases and a variety of endopetidases. Like animal enzymes, bacterial and mold proteases include enzymes which are active in acidic, neutral and alkaline pH. The majority do not require activation, but some are activated by reducing agents and some by metal ions such as Mn^{++}, Zn^{++}, and Co^{++}. A few inactive precursors have been found among the bacterial proteases. The proteolytic enzymes are generally classified into two groups, the proteinases (endo peptidases) which act only on inner peptide bonds of proteins and the peptidases (exo peptidases) which act on the peptide of bonds adjacent to the terminal amino acid. Several of the well characterized proteases of bacterial and mold origin have been shown to hydrolyze both proteins and many oligo peptides, and there are no microbial enzymes which have been clearly shown to be active only on proteins. In contrast to animal enzymes, all well characterized microbial protease, have very wide ranges of side chain specificity. One particular enzyme was shown to hydrolyze more than 80% of peptide bonds in proteins. Typical proteolytic enzymes are in the 10-35,000 molecular weight range.

Protein Degradation

Biological degradation and utilization of proteins was tested in batch reactors to establish the effects of protein concentration and to determine whether the addition of amino acids inhibits the degradation of proteins.

Innoculum was prepared in a continuous propagator using 100mg/l bacto-peptone feed solution. The innoculum was collected overnight and aerated continuously. Concentrates of bacto-peptone and glutamic acid were added at the beginning of each test to give concentrations in the range of 120-200 mg/l protein and 0- 90 glutamic acid.

The results of this test are summarized below:

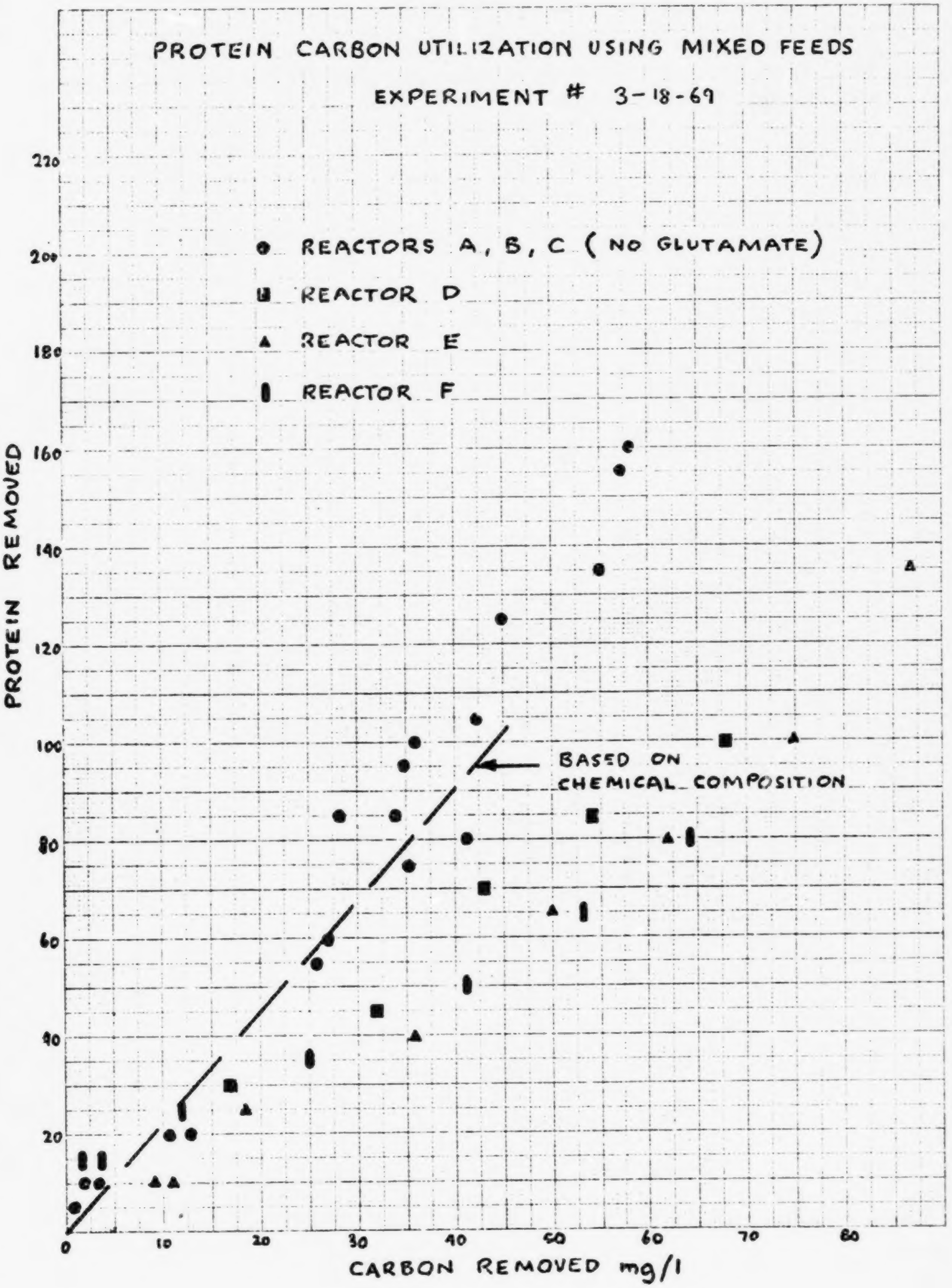
Initial concentration, mg/l			Growth Rate coefficient, min^{-1}	
Reactor*	Protein	Glutamic acid	Carbon data	protein measurements.
A	120	0	0.0077	0.0087
B	195	0	0.0087	0.0034
C	260	0	0.0087	0.0034
D	185	47	0.0095	- - - -
E	175	90	0.0101	- - - -

* Data from Exp. No. 3-18-69 in Appendix)

Growth rate coefficients were calculated using the simplified form of the batch reactor equation (zero order substrate kinetics). This approach is quite justified because the analysis was limited to the initial part of the test when the substrate concentrations were high. Comparison of the growth rates illustrate that the addition of glutamic acid increased growth rate somewhat, 10-20%. The increase in rate appears to be the result of incorporating glutamic acid into the cell growth metabolism. This is illustrated in Figure 18 which compares protein removal and carbon removal for batch tests which had received proteins only and mixtures of protein and glutamate. Batch tests with glutamate feed show a decidedly higher carbon uptake for a given removal of protein, indicating that glutamate is being removed from solution.

Glutamate acclimated innoculum was used to evaluate it capacity for degrading protein (peptone). Six tests were carried out using 50, 90 and 120 mg/l of protein and glutamate solution. The results show essentially no activity for utilization of protein, thus indicating that this innoculum had lost its hydrolytic activity through acclimation to glutamic acid as the only carbon and nitrogen source (Exp. No. 3-12-69).

FIGURE 18


PROTEIN CARBON UTILIZATION USING MIXED FEEDS
EXPERIMENT # 3-18-69
● REACTORS A, B, C (NO GLUTAMATE)
■ REACTOR D
▲ REACTOR E
▮ REACTOR F
BASED ON CHEMICAL COMPOSITION
PROTEIN REMOVED
220
200
180
160
140
120
100
80
60
40
20
0
10
20
30
40
50
60
70
80
CARBON REMOVED mg/l

A similar experiment using an innoculum that had not been operating as long on glutamic acid showed similar results, except that both glycine and peptone were eventually utilized though at a slow rate.

Substrate	Growth rate coefficient, min.$^{-1}$	Adsorption coefficient mg/l
Reactor A Glutamic acid	0.0043	20
Reactor B Glycine	0.002	34
Reactor C Peptone Glycine Glutamic acid	0.0001	- -

Observed value for peptone indicates that at the existing conditions, hydrolysis of proteins was repressed either by preventing the formation of hydrolytic enzymes or by inhibiting their funtioning.

Milk Solids

A number of investigators have used solutions of milk solids for process variable studies. This is one step closer to simulating municipal waste constituent as compared to the use of pure organic compounds and mixtures. Milk solids contain a large variety of organic materials such as proteins, carbohydrates and minor quantities of lipids as shown below:

	Analysis of Milk Solids - wt. %
Butterfat (lipids)	0.9
Protein	36.9
Lactose (carbohydrate)	50.5
Total solids	96.7
Organic solids	88.6
BOD (5 day)	75(mg/100 mg)
Organic Carbon	39.2

Milk solids acclimated innoculum was obtained from a continuous propagator using 100 mg/l feed solution. Concentrates of milk solids were added to each of 3 batch reactors and utilization of carbon, protein and carbohydrate were measured.

The results are summarized in Figure 19. Initial carbon removal is independent of the concentration of substrate but the rate slows down as substrate is depleted. Protein degradation proceeds rapidly and is essentially complete after 400 minutes. The residual concentration is probably the result of small peptides and interferences in the Folin test. It is interesting to note that carbohydrate removal is slower than protein degradation. Figure 20 shows the carbon data anaylzed in terms of the simplified batch rate equation; the results show that rates of removal are independent of concentration. Growth rate coefficient (μ) is 0.0016-0.0019 per minute and the adsorption coefficient (Kc) is in the range 18-31 mg/l. based on total carbon. If the non-biodegradable carbon is taken into consideration, the value of Kc is in the range 6-13 mg/l.

FIGURE 19

BIOLOGICAL DEGRADATION OF MILK SOLIDS

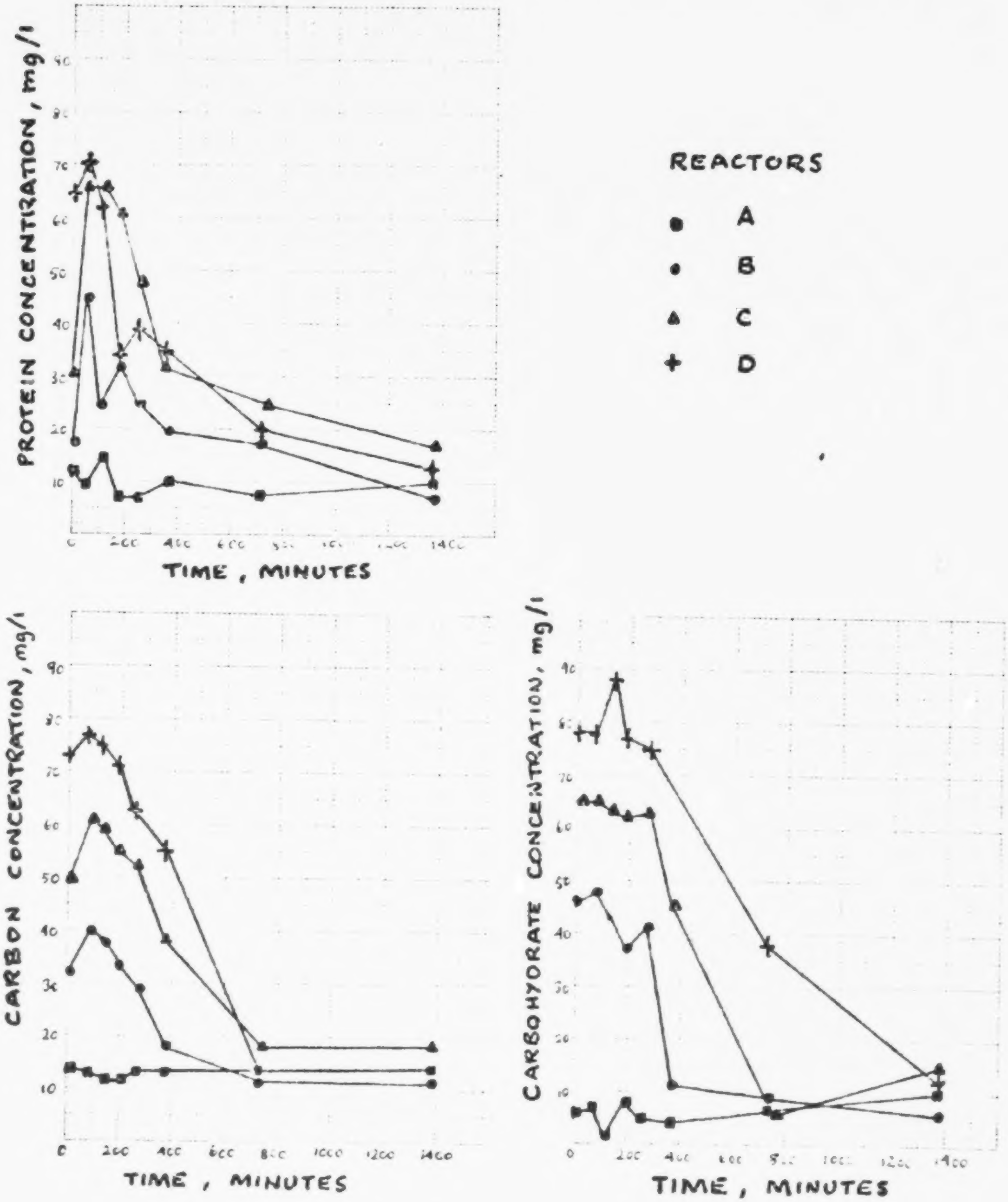
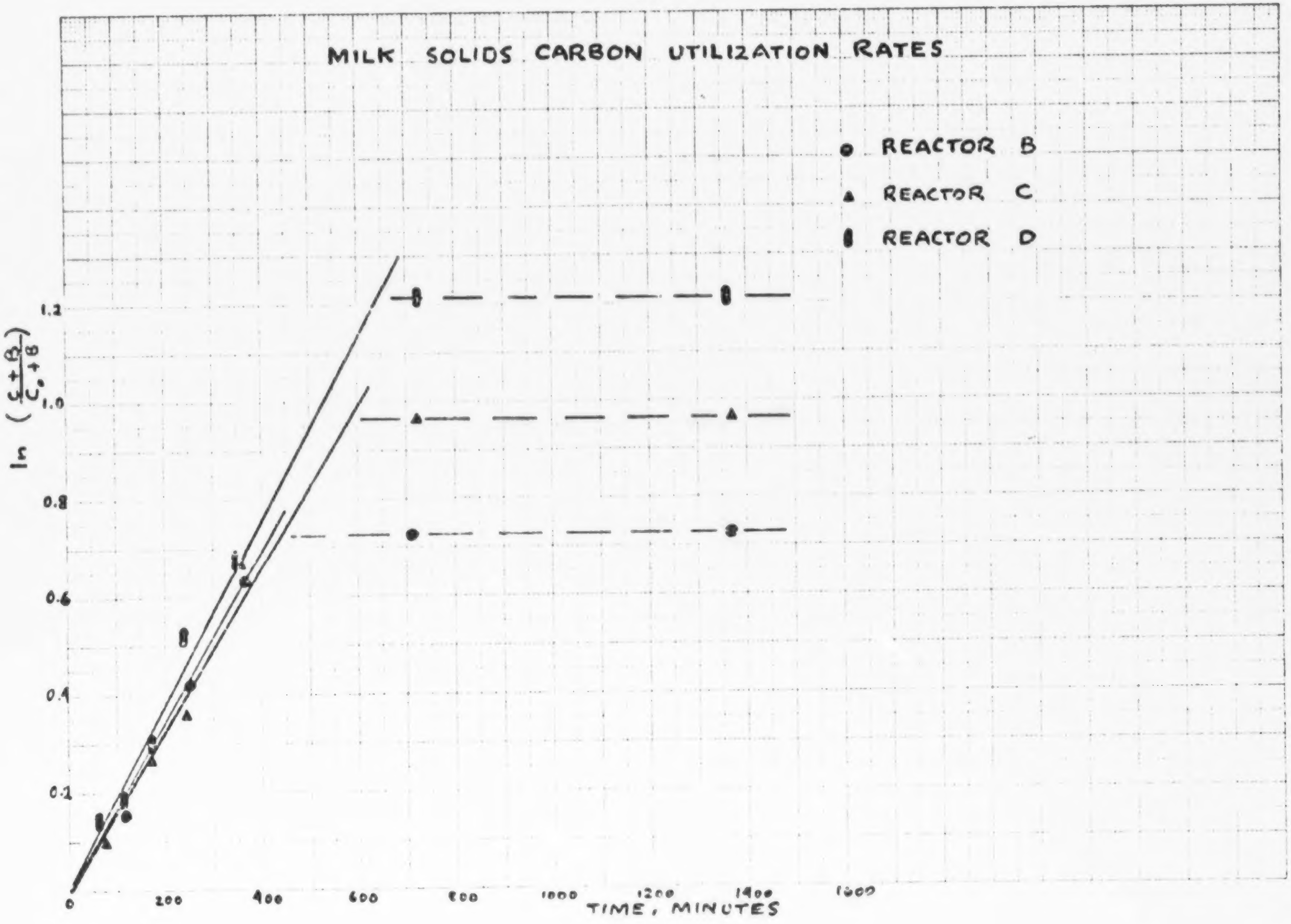


FIGURE 20


MILK SOLIDS CARBON UTILIZATION RATES
● REACTOR B
▲ REACTOR C
▮ REACTOR D
$\ln\left(\frac{C+B}{C_o+B}\right)$
1.2
1.0
0.8
0.6
0.4
0.2
0
200
400
600
800
1000
1200
1400
1600
TIME, MINUTES

Lipid Chemistry

The term lipid refers to a large group of organic compounds including fatty acids and their alcohol and sterol esters. The most prevalent lipids in municipal waste waters are the glyceride esters and the alkali salts of fatty acids.

The most significant chemical characteristic of lipids is their high carbon and hydrogen content and relatively low oxygen content. Where carbohydrates have 40-45 wt. % carbon, fatty acids have upwards of 70% carbon and very little combined oxygen. The result is that lipids have a much higher free energy content (available energy) than any other organic material. The yields of cell mass per unit weight of substrate are correspondingly high and the relative amounts of oxygen needed for complete oxidation (ultimate BOD) are much higher than those for carbohydrates and amino acids - proteins. Lipids are generally deficient in nitrogen, phosphorous and sulfur and thus resemble carbohydrates.

The role of microbial enzymes in transformation of lipids has been the subject of extensive research and publication (Microbial Transformations of Steroids, A handbook by Charney and Herzog, Academic Press 1967; Fatty Acids, Edited by Markley, Interscience Publishers, 1967). The action of microbial systems includes a wide variety of enzyme catalyzed reactions from oxidations and reductions to isomerization and hydrolysis.

> "As far as we know, transformations of steroids, carried out with intact microbial cells, occur within the cell and not in the medium surrounding the cell. To enter the cell the steroid being transformed must dissolve to some extent in the medium so that it can diffuse through the cell wall and into the enzyme-rich interior. The practical implication of this requirement is that solubility and rate of diffusion may become the rate-limiting factors for transformation. Most steroid substrates ordinarily employed have modest, though measurable, solubilities in water and in the aqueous media used for microbial culture. To ensure saturation of the medium and to minimize this rate-limiting effect, steroids are often introduced into reactions in micronized form or, more conveniently, in solution in a water-miscible solvent from which precipitation in very fine particles occurs upon dilution with the aqueous medium containing the microorganisms.
>
> The experimental findings may be interpreted reasonably to show that microbial enzymes are not highly substrate specific. The alternate explanation for the diversity of substrates which a given species can transform is that the organism has a different enzyme for each new substrate. The latter explanation is much less satisfying, and no evidence has been adduced in its support."

One possible explanation for the nonspecificity of microbial enzymes is that microbial organisms are also producers of lipids internally and therefore requires the capability to catabolize lipid materials in the natural growth cycle.

The hydrocarbon chain and the carboxyl group are the most prominent characteristics of lipids. They impart special physical characteristics which make for low solubility and surface activity in water solutions. Solubility in water is a strong function of molecular size, pH and salt concentrations.

Solubilities of Fatty Acids in Water
Grams acid per 100 g. water

Acid	0°C	20°C	30°C	45°C	60°C
Caproic	0.864	0.968	1.019	1.095	1.171
Heptanoic	0.190	0.244	0.271	0.311	0.353
Caprylic	0.044	0.0068	0.079	0.095	0.113
Nonanoic	0.014	0.026	0.032	0.041	0.051
Capric	0.0095	0.015	0.018	0.023	0.027
Hendecanoic	0.0063	0.0093	0.011	0.013	0.015
Lauric	0.0037	0.0055	0.0063	0.0075	0.0087
Tridecanoic	0.0021	0.0033	0.0038	0.0044	0.0054
Myristic	0.0013	0.0020	0.0024	0.0029	0.0034
Pentadecanoic	0.00076	0.0012	0.0014	0.0017	0.0020
Palmitic	0.00046	0.00072	0.00083	0.0010	0.0012
Heptadecanoic	0.00028	0.00042	0.00055	0.00069	0.00081
Stearic	0.00018	0.00029	0.00034	0.00042	0.00050

(*J. Org. Chem.*, 7, 546, 1942)

Substantially higher solubilities of the long carbon chain fatty acids are obtained in alkaline solution by salt formation. (saponification).

Lithium Salts	gm/100 gm H_2O (25°C)
Lauric	0.187
Myristic	0.036
Palmitic	0.015
Stearic	0.01

It has been observed that a number of the higher molecular weight fatty acids can be dispersed in water in excess of their reported solubility and maintained in suspension. One mechanism for such dispersion is the formation of a thin film at the air-water interface, (frequently assumed to be a monomolecular film). This in effect makes the air-water interface a rich source of organic material and oxygen for those microorganisms that metabolize fatty acids. Another phenomenon which enhances the "apparent" solubility of fatty acids is the formation of micelles. Micelles are aggregations of molecules to form colloids. According to Jirgenson, micelles are formed by molecules with large hydrophobic parts and a strongly polar group in the same molecule. (Organic Colloids, Jirgenson, p.50, Elseier Press 1958). Arrangement of molecules is believed to be such that the long carbon skeletons are parallel to each other with the carboxyl group extending into the solution in order to minimize water contact. This type of behavior is evidenced by decreasing osmotic activity at increasing concentrations of the hydrophobic material. Micelle formation is dependent on several variables, including concentration of the molecular species. Critical concentration which leads to micelle formation have been reported.

Potassium laurate	0.02 moles/l. (26°C)
Potassium Linoleate	0.0002 moles/l. (26°C)

Fatty Acid Degradation

Three fatty acids were tested, lauric, palmitic, and stearic. Feed solutions were prepared by solubilizing the fatty acid in approximately 200 ml. of 1.0 normal sodium hydroxide solution. This concentrate was then dispersed rapidly into feed solution (5 gallon batch) which contained all the necessary salts. The pH was adjusted by adding hydrochloric acid. The resulting solutions frequently developed a cloudy appearance, indicating that there was some formation of colloidal dispersions (micelles).

In order to test for colloid formation, the feed solutions were occasionally filtered through millipore filters. It became apparent that at the desired concentrations, up to 100mg/l, only lauric acid solution remained in reasonable dispersion. As a result, most of the biodegradation studies were carried out with lauric acid.

Considerable effort was expended on developing or adapting analytical procedures for the precise determination of fatty acids at the concentrations in question. (0 to 100 mg/l). Unfortunately, the available methods are not sufficiently sensitive at low concentrations. Furthermore, extractions with fat specific solvents were not quantitative nor sufficiently reproducible to allow measuring the small changes in fatty acid content that are obtained in kinetic studies of this type. As a result, it was necessary to calculate or estimate fatty acid degradation from carbon removal data and specific analysis for the other substrates in order to back calculate removal of fatty acids.

Biological uptake of lauric acid was measured in batch tests using innoculum from a continuous propagator using 50 mg/1 lauric acid feed as the only source of carbon. The innoculum was thus well acclimated to metabolizing the lauric acid substrate. Different amounts of fresh feed solution (50 mg/1) were added at the start of each batch test, and samples removed periodically for carbon analysis (Experiment No. 10-7-69) and 10-18-69). The results show that acclimated innoculum is an effective biological system for removing fatty acids as illustrated in Fig. 21 which shows the removal of carbon as a function of time. The results were correlated using the simplified batch reaction equation (assumes adsorption coefficient K is negligible). The calculated growth rate coefficient is 0.0037 per minute (25°C), carbon residue was approximately 6 mg/1 and corresponds to 15 wt. % of the initial feed carbon. Higher growth rates were obtained in batch tests which started with higher dilution ratios of feed to innoculum, up to 0.006 per minute, (Exp. No. 12-18-69 and 12-23-69) reason for this is not known. It is unlikely that substrate concentration is responsible because the calculated asdorption coefficient (Kc) from these batch tests on fatty acids is quite low, 7-16 mg/1. One possible explanation is that the fatty acids are removed from solution-suspension by adsorption on the biomass surface and are therefore not as readily available for ingestion into the microbial cells.

A similar series of batch tests (Exp. No. 12-23-69) using palmitate acclimated innoculum shows similar results. The calculated growth rate for these tests in the range of 0.0057 - 0.0064 and the adsorption coefficient K is 7-16 mg/1. The average value of the calculated yield of cell mass is 95 wt. % based on carbon removed. The carbon residues were very low, indicating that substrate was almost completely removed from solution. Initial rate data using palmitic acid feed solution may not be reliable because there is some evidence that palmitate was removed by the millipore filters prior to analysis. Innoculum which was acclimated to lauric acid feed (50 mg/1) was contacted with feed concentrates of glucose and glutamic acid in a series of batch tests (Exp. No. 12-30-69 and 1-29-70). The results show that the microbial population is capable of metabolizing these substrates without any difficulty and without any lag. Figure 22 shows the removal of carbon; the initial rate is independent of total carbon concentration and substrate added. The initial growth rate coefficient is 0.0065 per minute. However, it is interesting to note that the calculated values of Kc are higher for the batch tests which had received glucose and glutamic acid, 6 mg/1 for Reactor A using only laurate and 40-50 mg/1 for the mixed feeds. This suggests that the lauric acid acclimated microorganism population does not proliferate as well where lauric acid is no longer available. Further evidence for this is shown in Figure 23 which summarizes the data for removal of glucose and glutamic acid measured independently. It is interesting to note that the removal of glutamate begins 200 minutes after the start of the test and glucose

FIGURE 21

FATTY ACID DEGRADATION - CARBON REMOVAL

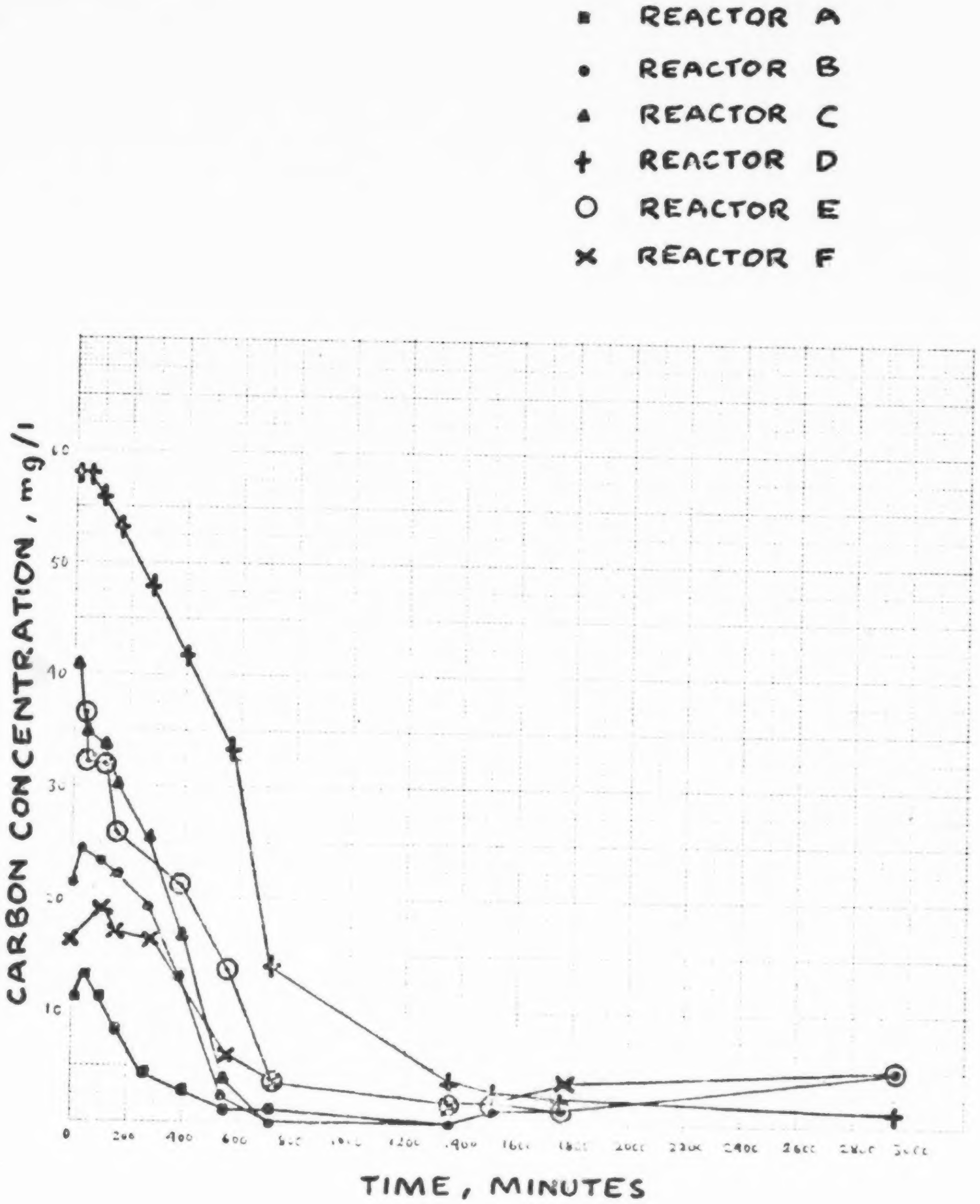


FIGURE 22

DEGRADATION OF MIXED FEED SOLUTION BY

LAURATE ACCLIMATED INNOCULUM

	REACTOR	FEED
■	A	LAURIC ACID
●	B	LAURIC + GLUCOSE
▲	C	LAURIC + GLUTAMATE
+	D	LAURIC + GLUCOSE + GLUTAMATE

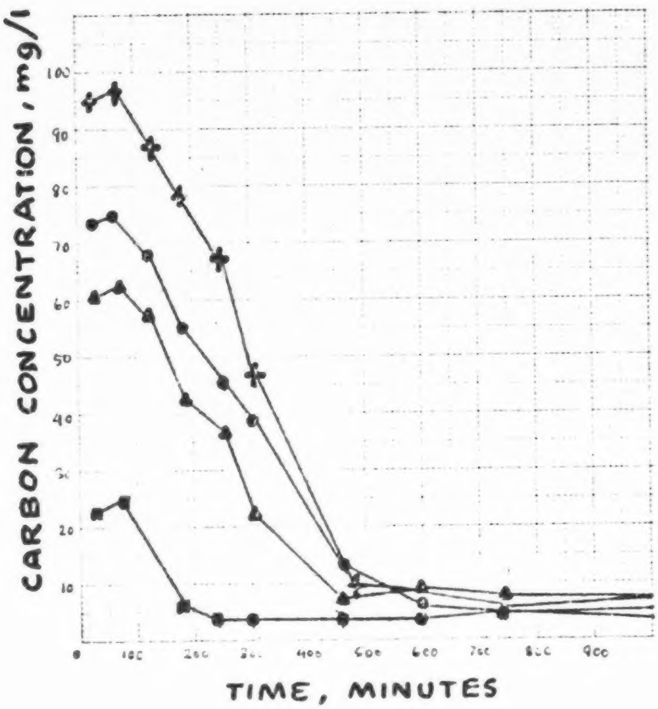
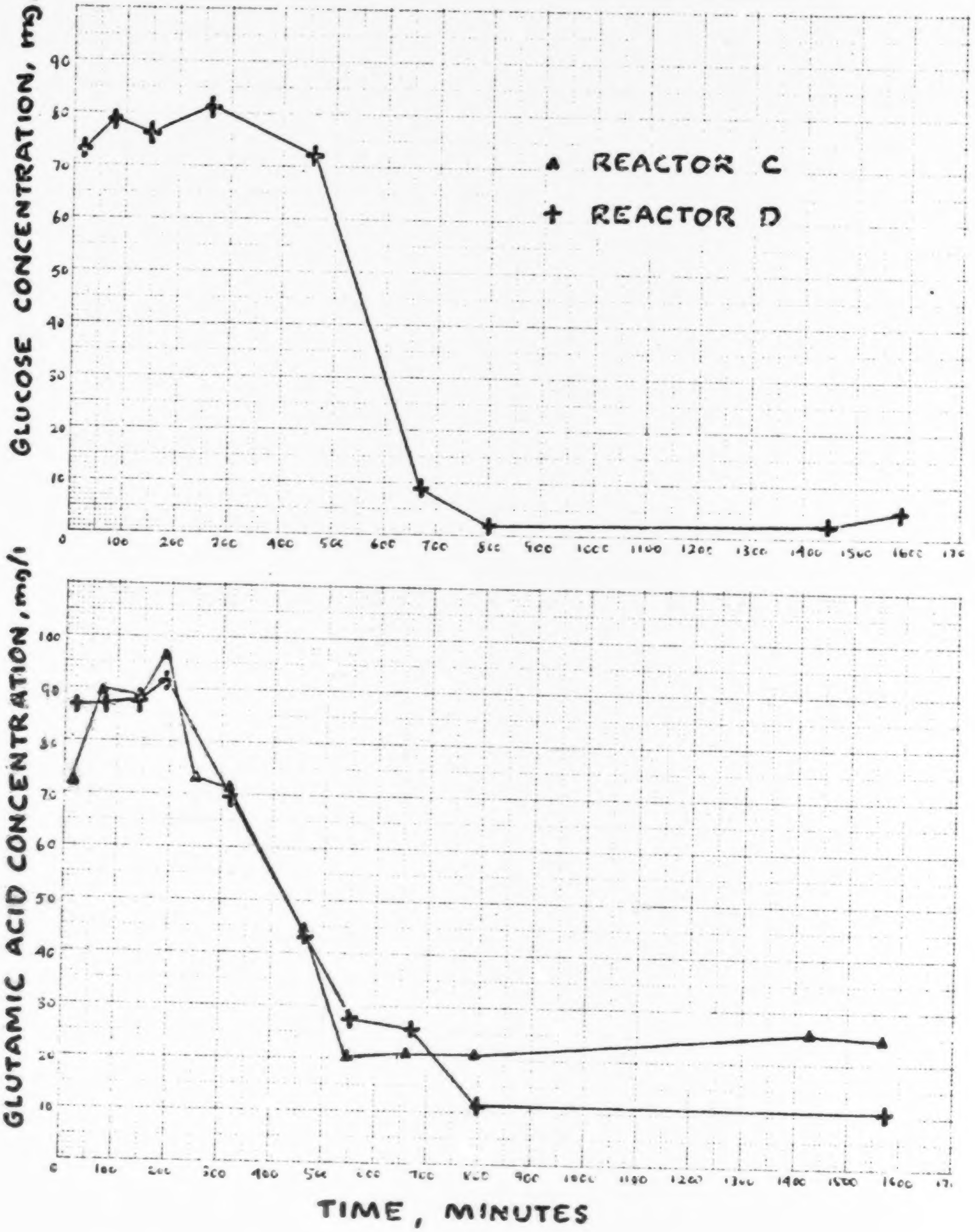


FIGURE 23

REMOVAL OF GLUTAMATE AND GLUCOSE BY LAURATE ACCLIMATED INNOCULUM



removal is delayed for 300-400 minutes. Thus lauric acid was removed preferentially and the other substrates were utilized only after most of the lauric acid substrate had been consumed. The utilization of glutamate before glucose is especially noteworthy. The batch tests were fed with a nitrogen limited solution so that this may account for some preferential uptake of glutamate, however, it is also evident that glucose was not used until most of the glutamic acid was degraded so that the nitrogen deficiency is not the whole answer.

Additional tests using innoculum from a propagator using mixed feed solutions were carried out (Exp. No. 2-23-70, 4-30-70, 6-19-70, and 7-15-70). Concentrates of lauric, glutamate and glucose were added at the start of each batch test. To overcome the nitrogen deficiencies observed in some tests, Exp. No. 6-19-70 and 7-15-70 were modified to include a source of ammonium ions when glucose or laurate concentrates were added. Figure 24 shows the removal of carbon when mixed feed innoculum is exposed to lauric acid, and glutamic acid alone and in admixture. Initial rates of removal are essentially equivalent; carbon concentration drops off to approximately 30 mg/l in less than 600 minutes except for the mixed substrate. It is also noteworthy that the residual carbon level remains high (30 mg/l) even after 100 hours. Comparison of the rates of glucose, and glutamic acid removal show that the latter is used preferentially over glucose, Figure 25. This holds for the pure substrate tests as well as the mixed feed test. Furthurmore, the removal of lauric acid is at least as fast as the other substrates. The more gradual removal of carbon from the mixed feed test finds a parallel in the more gradual removal of glucose shown in Figure 25.

FIGURE 24

MIXED FEED INNOCULUM

CARBON REMOVAL

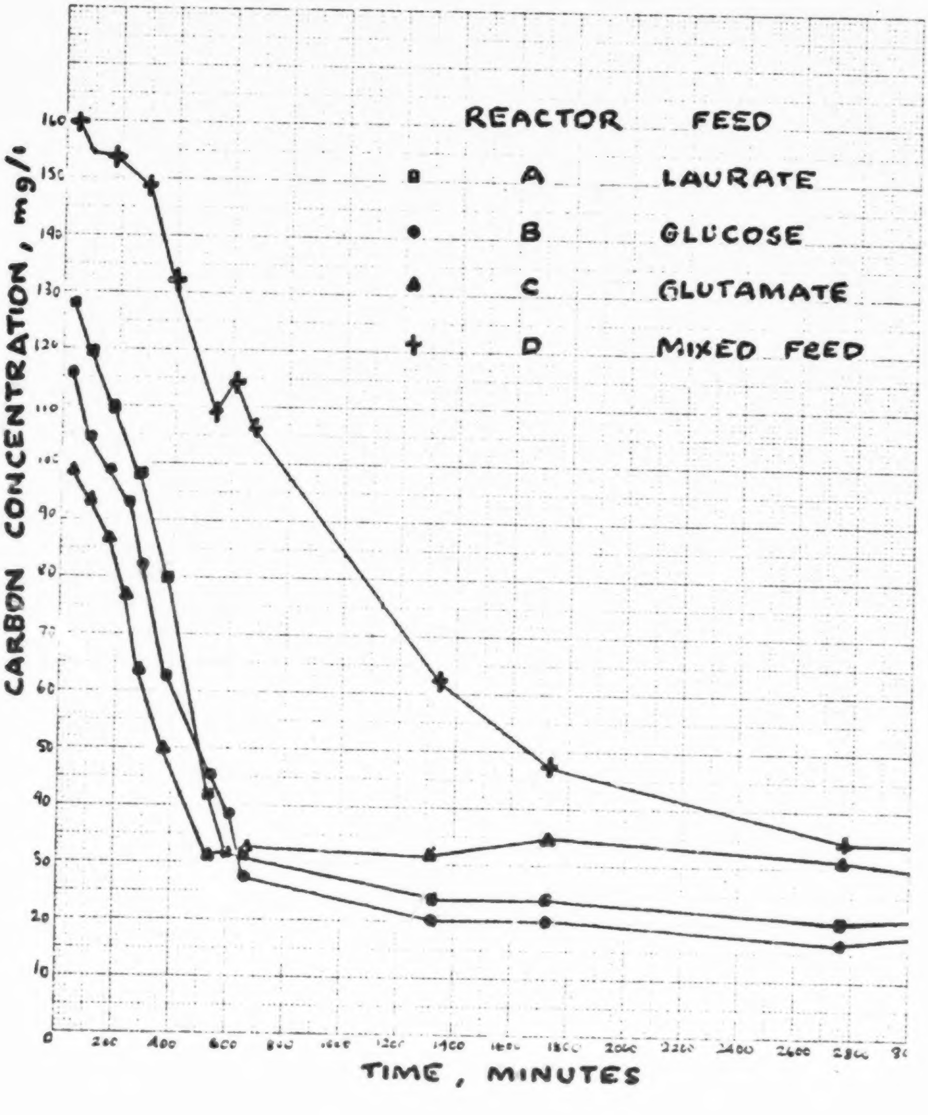
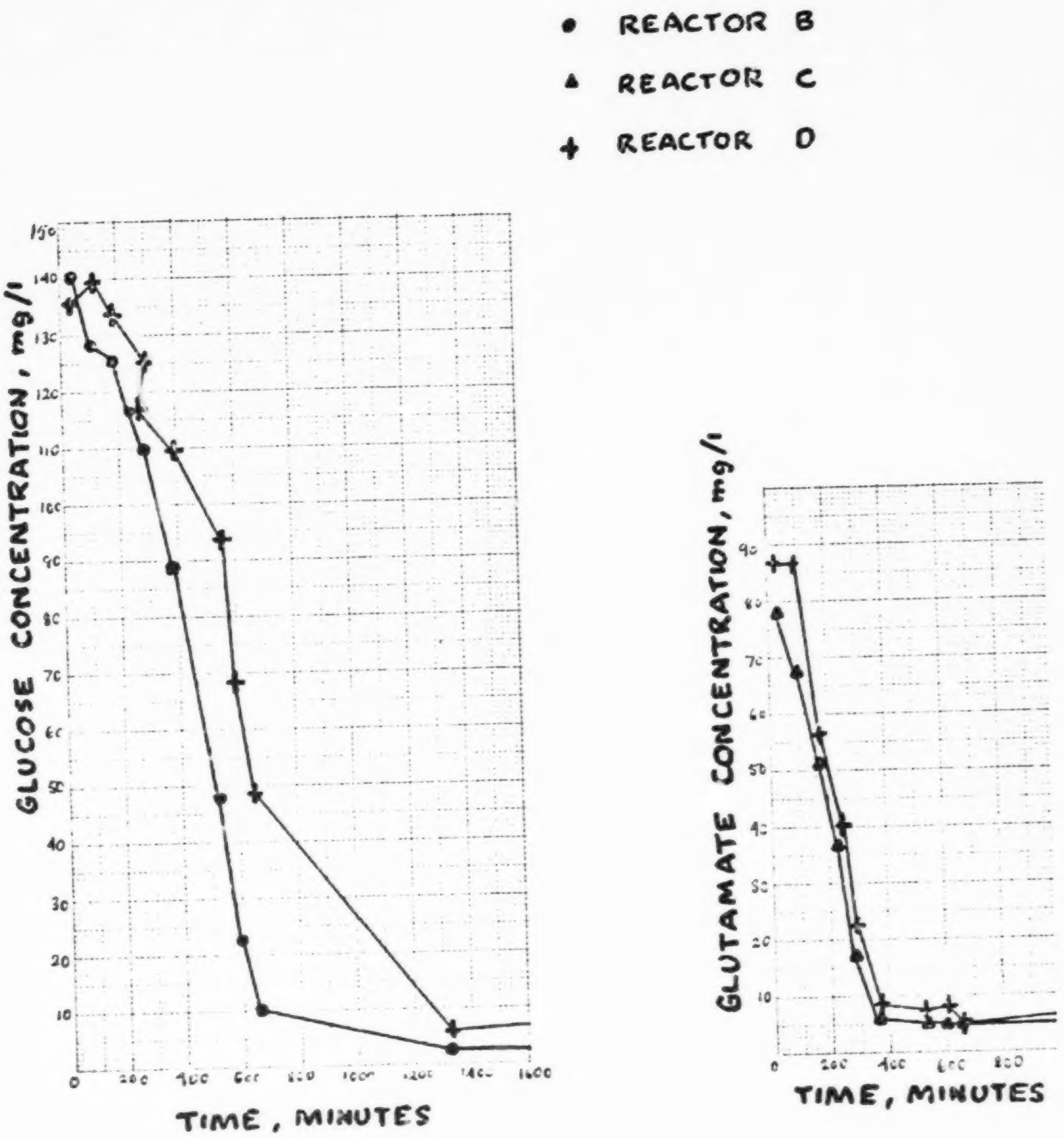


FIGURE 25

MIXED FEED INNOCULUM

GLUCOSE AND GLUTAMATE REMOVAL



Rate Studies Using Treatment Plant Biomass

A series of batch tests were carried out using activated sludge from the Minneapolis-St. Paul Sewage Treatment Plant.

The tests were designed to evaluate the activity of activated sludge biomass and to measure the relative rates of removal of the three major categories of substrate, namely carbohydrates, proteins and lipids, (Exp. No. 7-23-70 and 8-11-70). Activated sludge was taken at the plant just prior to the tests. Concentrates of various substrates were added at the start of each test. Samples were withdrawn for analysis as previously described. Experiment No. 8-11-70 consisted of 7 batch tests each starting with 200 ml of dilute activated sludge (492 mg/l suspended solids). Concentrates of pure substrates were added as shown below.

Volumes of Concentrate Added*, ml.

Glucose	Starch	Glutamate	Peptone	Laurate	Reactor
40	- - -	- - - -	- - -	- - -	A
- - -	40	- - - -	- - -	- - -	B
- - -	- - -	40	- - -	- - -	C
- - -	- - -	- - - -	40	- - -	D
- - -	- - -	- - - -	- - -	160	E
20	- - -	20	- - -	40	F
- - -	20	- - - -	20	40	G

*1000 mg/l except for laurate which was 100 mg/l as lauric acid.

Comparison of the concentration versus time profiles of each substrate and carbon gives some insight on the relative rates of removal and the ability of the biomass to handle single substrates in large doses. A second batch of concentrate was added after 400 minutes and substrate removal measured for an additional 1000 minutes.

Analysis of the carbon data shows that glucose was removed more rapidly than any other substrate. Glutamate was removed rapidly after the second addition of concentrate but showed a lag during the first part of the test. The most rapid removal of carbon was noted with the mixture of glucose-glutamate-laurate. By contrast, degradation rates of the colloidal substrate, starch and protein, were slower in both parts of the test. Data for glucose removal are shown in Figure 26. Glucose is removed completely and at a constant rate from the beginning of the test. Figure 27 shows the corresponding data for amino acids. Removal rates are more variable. The gradual increase in amino acid concentration in Reactor G is probably the result of hydrolytic degradation of proteins to form amino acids which are subsequently removed by the biomass. Removals of starch and protein are summarized in Fig. 28 and 29 respectively. The rates of removal after the second addition of concentrate are higher in each case, suggesting that the sludge as received is not sufficiently acclimated to hydrolyze colloidal substrates at high rates. The slow rate of colloid degradation seems surprising because municipal sewage has some 50% suspended material of which half is settleable and half is in the colloidal size range. One possible explanation for the hydrolytic activity may be the prior history of the recycled sludge biomass. Free exoenzyme being relatively small colloids, molecular weights of 100,000 or less, are more likely to be carried out with the effluent from the final sedimentation tank. The hydrolytic enzymes associated with the cell surface are frequently subjected to a period of endogenous metabolism in the final sedimentation tank and in the latter part of the aerator itself where the detention time is long.

FIGURE 26

GLUCOSE REMOVAL BY ACTIVATED SLUDGE BIOMASS

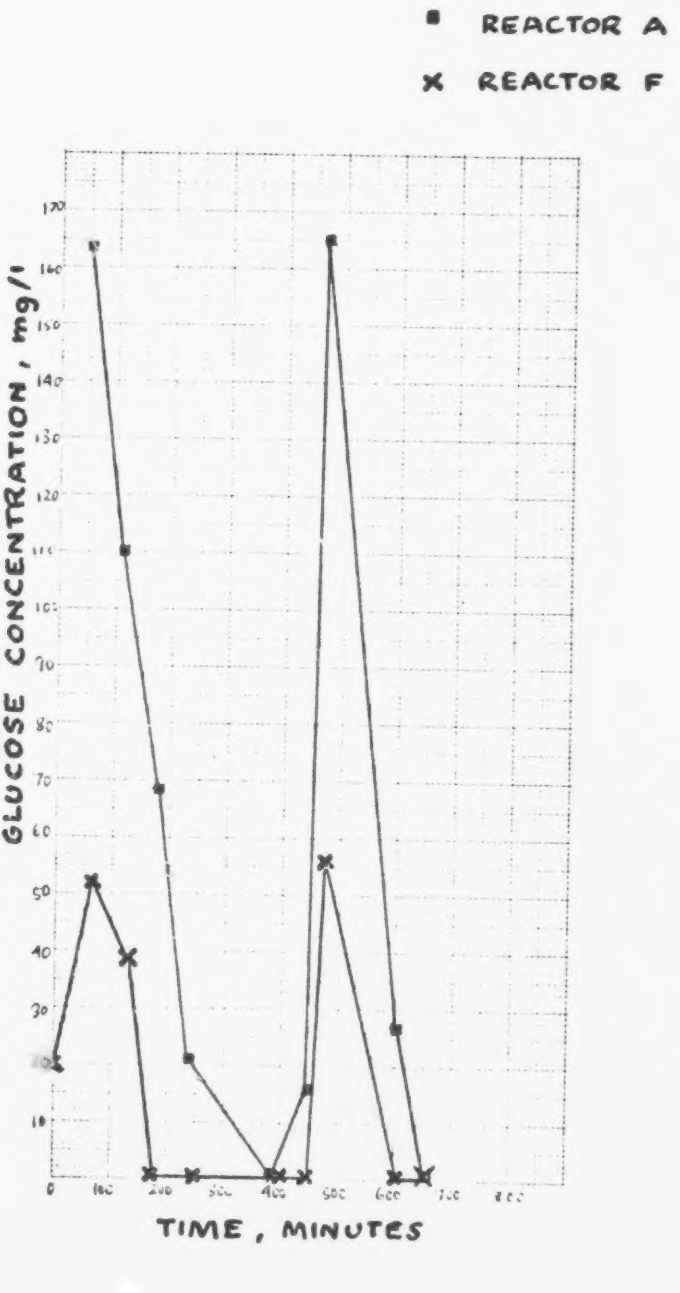


FIGURE 27

AMINO ACID REMOVAL BY ACTIVATED SLUDGE BIOMASS

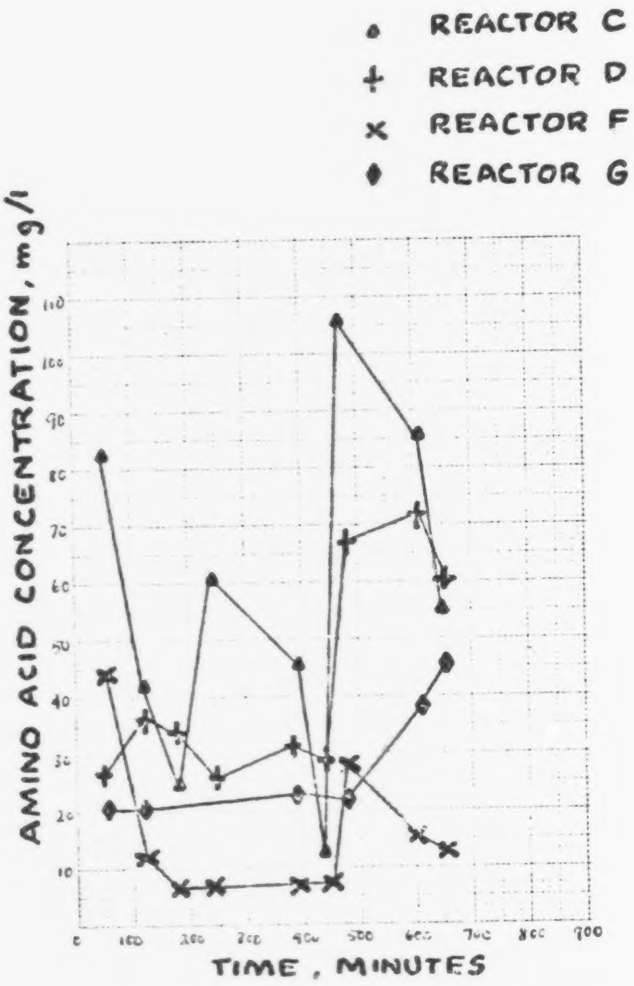


FIGURE 28

STARCH REMOVAL BY ACTIVATED SLUDGE BIOMASS

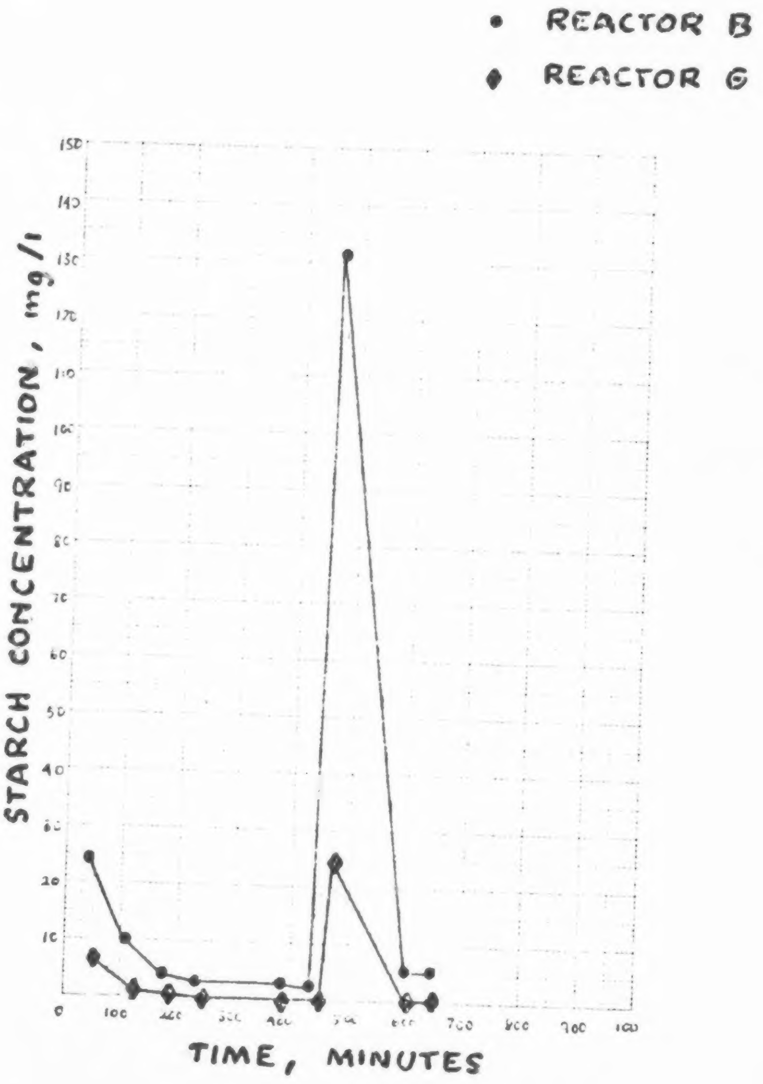
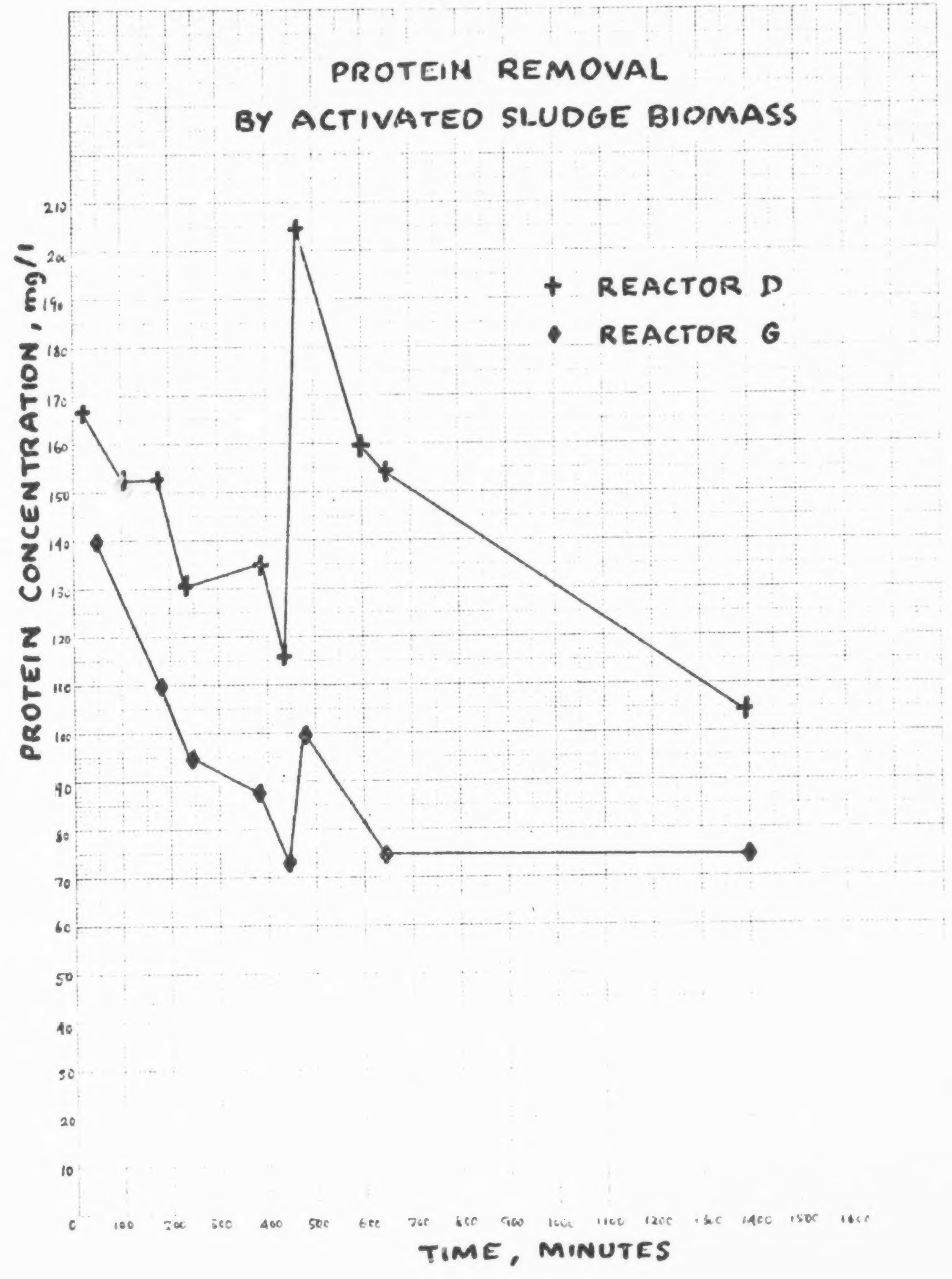


FIGURE 29



PROTEIN REMOVAL
BY ACTIVATED SLUDGE BIOMASS
+ REACTOR D
♦ REACTOR G
PROTEIN CONCENTRATION, mg/l
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
TIME, MINUTES

Measurement of the Rate of Oxygen Uptake of Sewage Solids

This experiment was designed to measure the rate of oxygen uptake resulting from the addition of typical raw sewage solids to a typical mixed liquor activated sludge innoculum. Parallel experiments were carried out to measure oxygen uptake using mixtures of soluble substrates (glucose and glutamic acid) and colloidal substrates (peptone and starch) and aliquots of the same innoculum. Tests were carried out in a 1.5 liter enclosed vessel. Oxygen determinations were made using a dissolved oxygen electrode probe; samples were analyzed for organic carbon.

The sewage solids were obtained by centrifuging primary effluent from the Minneapolis-St. Paul Treatment Plant. The solids were collected and autoclaved for 10 minutes and stored under refrigeration until use. Prior to the test the solids were treated in a blender for about 5 minutes to disperse large flocculated material and to reduce particle size somewhat. Feed concentrates of glucose, glutamic acid, peptone, and starch were also autoclaved prior to use.

At the beginning of each test, sewage solids or fresh feed solutions were added as shown below:

Reactor A - 2.0 liters mixed liquor; no feed added, mixed liquor suspended solids concentration was 1,330 mg/l.

Reactor B - 1.5 liters mixed liquor, added 0.25 liters each of 1600 mg/l concentrate of glucose and glutamic acid, mixed liquor suspended solids concentration was 890 mg/l.

Reactor C - 1.5 liters mixed liquor, added 0.25 liters each of 1600 mg/l concentrate of peptone and starch, mixed liquor suspended solids concentration was 875 mg/l.

Reactor D - 1.5 liters mixed liquor, added 0.5 liters of sewage solids obtained from centrifugation of raw sewage, mixed liquor suspended concentration was not determined after solids addition but was calculated to be 860 mg/l based on the innoculum alone. The solids alone had a suspended solids concentration of 2,715 mg/l.

Reactor E - 2.7 liters of mixed liquor from composite of reactors B, C, and D, added 0.1 liters each of 900 mg/l glucose and glutamic acid solution.

Reactor F - 2.7 liters of mixed liquor from composite of reactors, B, C, and D, added 87.5 ml. of sewage solids.

The mixtures were aerated and agitated continuously except for the time during which oxygen measurements were carried out. The mixture was transferred into the special reactor and oxygen determinations were made continuously using a strip chart recorder. The rate of oxygen consumption was then determined from the strip chart recordings. Samples were also removed and total organic carbon measured for each oxygen consumption test.

Oxygen uptake rate expressed as milligrams of oxygen consumed per hour per gram of suspended solids as a function of time in minutes are shown in Figure 30, the corresponding organic carbon concentrations are shown in Fig. 31. Reactor A represents the base case mixed liquor activated sludge without any extraneous addition of fresh substrates. The oxygen consumption rate remains fairly constant at about 10 milligrams per hour per gram of suspended solids. By constrast both reactors B and C show a marked peak after the addition of fresh substrate. Oxygen uptake in Reactor C using collodial substrates (Peptone and Starch) are approximately the same as for Reactor B using soluble substrates (glucose and glutamic acid). This suggests that mixed liquor contains sufficient concentrations of hydrolytic enzymes to convert colloidal materials into assimilable soluble substrates. Reactor D which received the sewage solids additionally showed the highest oxygen uptake rate. This is particularly surprising in view of the fact that the dissolved carbon of Reactor D was if anything somewhat lower than Reactor C. One possible explanation for this observation is that the sewage solids contain a broad range of substrates which favor growth of a large variety of microorganisms, such as are found in sewage. By contrast it may be that specialized substrates, such as pure compounds, may be restrictive in the species that can thrive immediately on the limited substrate variety.

Test results using mixed liquor from an activated sludge plant as innoculum show that there is sufficient enzyme activity to degrade colloidal materials (as measured by oxygen uptake). This conclusion is based on the observation that the addition of colloidal substrate gave approximately the same rate of increase in oxygen uptake as when soluble substrates were added. The initial dosing of food materials is consumed during the first few hours but then levels off leaving a large residue of organic carbon in solution. Oxygen uptake rate also levels off but continuous for more than 20 hours without any substantial changes in dissolved organic carbon. The results indicate that continuous aeration leads to endogenous respiration of the cell mass but does not reduce the dissolved organic carbon concentration of the liquor to any appreciable extent during the first 20 hours or more.

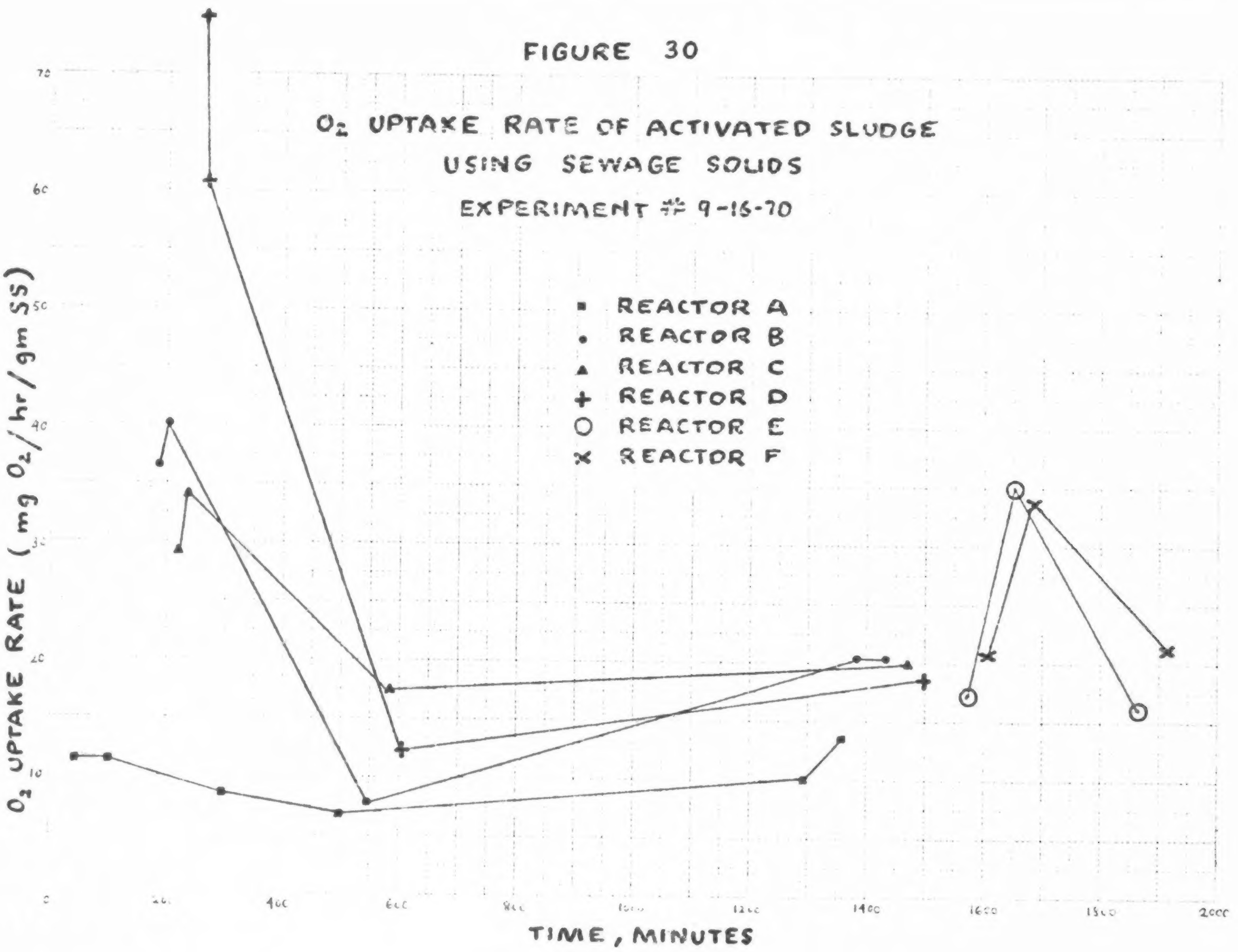
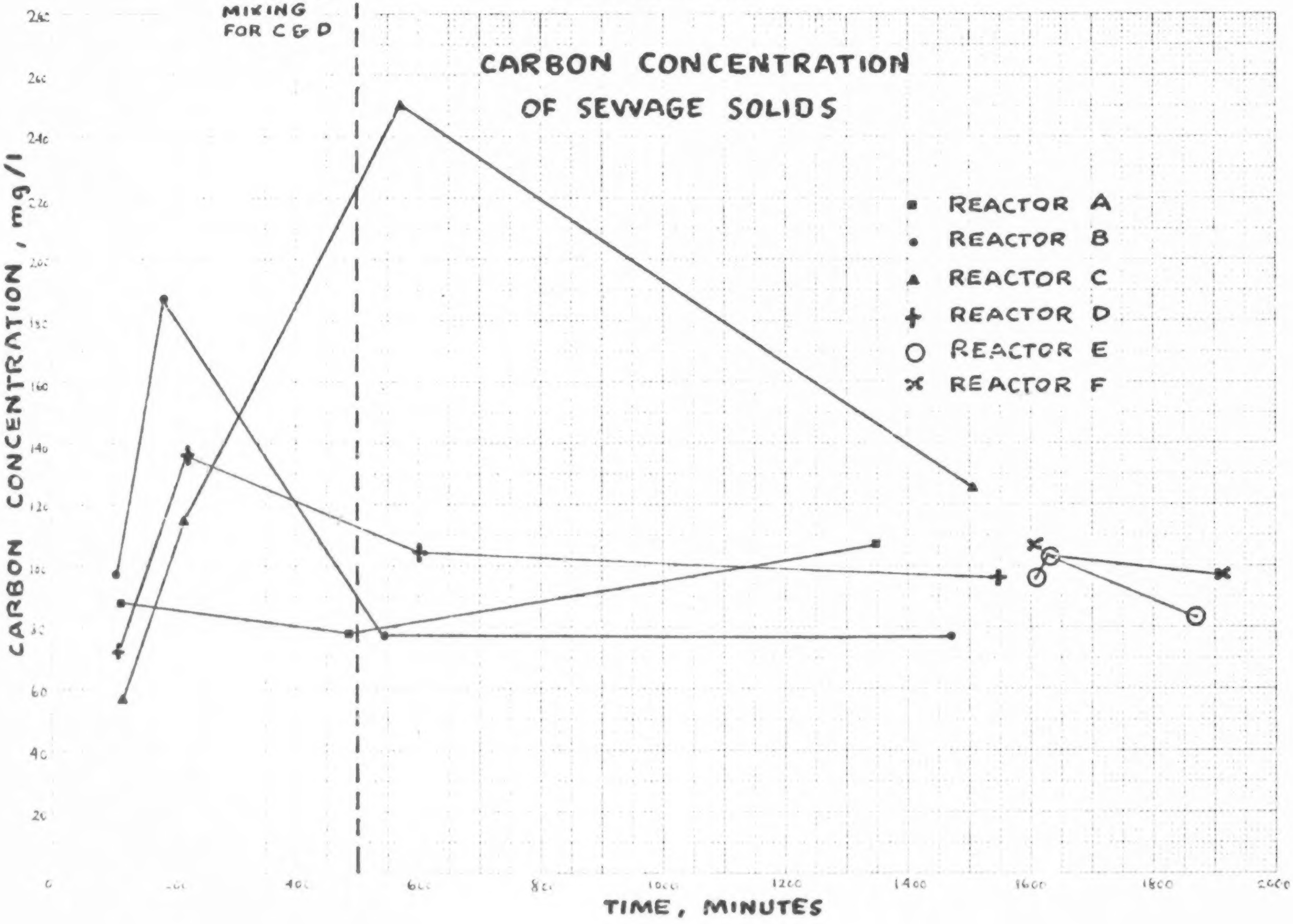


FIGURE 30

O₂ UPTAKE RATE OF ACTIVATED SLUDGE USING SEWAGE SOLIDS

EXPERIMENT # 9-16-70

FIGURE 31



CARBON CONCENTRATION OF SEWAGE SOLIDS

FILM FLOW REACTOR TEST RESULTS

There is growing interest in the quantitative description of biologically mediated reactions in connection with waste water treatment and natural purification of waters. Most of the published data in this field concerns well mixed reaction systems in which the active cell mass is suspended in the growth medium by agitation. Relatively little information is available on the kinetics of stationary, biologically active surfaces (slime layers). Stationary slime layers are important in at least two areas; namely, in the trickling filter process for sewage treatment and in the attached biomass of stream beds.

This section describes results of studies using fixed biologically active slime layers to determine the kinetics of removal of organic substrates. The substrates were chosen with a view to simulating the major constitutents of domestic waste water; namely, carbohydrates, amino acids-proteins, and fats. The immediate objective is to provide a better understanding of the trickling filter process in sewage treatment. However, the approach and results may be of general interest in biological processing.

MASS TRANSFER CONSIDERATIONS IN BIOLOGICAL TREATMENT

It is generally recognized that there are many similarities between enzyme kinetics and heterogenous chemical reaction kinetics. Reactants, intermediate adsorbed species and product species can be defined and measured. By contrast, the biological activity of whole microorganisms is much more complex. Microbial metabolism is the sum of thousands of enzyme catalyzed reactions, simultaneous and sequential, dependent and independent of each other. Nevertheless, it has been found useful to apply some of the concepts of heterogenous catalysis to evaluate biological kinetics; namely, mass transport of substrate to the active sites and adsorption and reaction at the surface. In heterogenous catalysis, mass transfer limitations are likely to occur when, (a) concentration of reactants is low, (b) catalytic sites are inaccessible to bulk flow, and (c) potential reaction rate is high. Liquid phase reaction systems are frequently limited by mass transfer because diffusional transfer through the boundary layer is very slow. Some of the same conditions prevail in biological waste water treatment systems such as trickling filters and to a lesser extent in the floc particles in activated sludge treatment.

A number of investigators have shown that biological reaction rates are limited by the availability of either substrate, oxygen, or salts. Rashevsky (*Mathematical Biophysics*; volume I; page 36, Dover Press) has described an analytical model for a spherically shaped microorganism immersed in a bulk fluid. The model describes concentration gradients in the exterior boundary layer of the cell assuming that the layer is stagnant and mass transfer is by molecular diffusion. Mueller, Boyle, and Lightfoot, (21st Purdue Industrial Waste Conference, May 1966) studied the effects of floc size in a well mixed agitated reaction vessel to simulate the conditions obtained in activated sludge aeration tanks. For dispersed cells of Zooglea ramigera, minimum oxygen concentration for uninhibited growth was found to be 0.1 mg/1. For floc particles, minimum O_2 concentration was 0.6-2.5 mg/. depending on floc size. Resistance to oxygen mass transfer in the liquid film surrounding floc particles was negligible compared to the resistance within the clump of cells.

Studies using fixed slime layers to simulate some aspects of trickling filter operations have been reported by several investigators. Gulevich (Johns Hopkins University Report, Jan. 1967) used rotating disks covered with biological slime and immersed in feed medium. Glucose uptake rate was found to be a function of the velocity field. Hartmant (J. Wat. Poll. Control Fed., 39, 958, June 1967) used a tubular reactor coated with biological slime and measured the effects of flow rates on oxygen consumption. The results show that increasing the velocity increased oxygen consumption, except at very high flow rates. He ascribes this to increased mass transfer from the bulk fluid to the slime layer. Swilley (Rice University Report, Feb. 1965) and Maier (San. Eng. Div., ASCE, SA4, p.91, Aug. 1967) used a flat surface slime layer to study biological uptake of glucose from medium percolating over the slime in a thin film. These studies have demonstrated that mass transfer limits uptake at low concentrations. The slime layer-film flow reactor data have been considered in the light of analytical models patterned after the film flow reactor models used in heterogenous catalysis. A derivation and solution for the flat plate reactor has been published by the author (Thesis, Cornell University, Sept. 1966) and will not be repeated here. The model allows making predictive statements about the maximum amount of substrate available at the slime-liquid interface as a function of flow rate, slope of surface, substrate concentration in the bulk liquid phase and substrate type. This model has also been used as a reference point for the studies to be described below.

BIOLOGICAL SLIME LAYER

Most of the literature discussion of slime layers is with reference to trickling filters. Holtje (Sew. Works Journ., 15, 14, Jan. 1943) has described the development and species composition of slime layers in trickling filters. Typically, the slime consists of large numbers of microorganisms (3×10^8 cells/cm^3); a large variety of microorganisms, namely bacteria, fungi, protozoa, and higher forms of life have been identified. Popluation distribution and density reflects environmental conditions such as composition and concentration of wastes, temperature, and hydraulic loading. Surface growth starts with the attachment of zoogleal bacterial at the solid surface. Wattie (*Sew. Works Journ., 15,* 476, 1943) has described the cultural characteristics of pure cultures isolated from trickling filter slimes; they are gram negative short rods, enmeshed in a gelatinous mass. Their characteristics are similar to *Zooglea ramigera* as described in Bergey's Manual of Determinative Bacteriology. Hawkes (*The Ecol. of Waste Water Treatment,* McMillan Co., 1963) has published the most comprehensive description of the ecology of slime layers. It has been suggested that bacterial slimes form a gelatinous matrix which acts as a binder and holds the bacteria together. The slime layer thus provides a habitat for other species. Filamentous forms such as *Sphaerotilus* and *Beggiatoa* are frequently present. Nitrite and nitrate producing bacteria build up, given sufficiently long residence time. The occurrence of fungi depends on operating conditions. Since bacteria and fungi are in competition for soluble substrates, the bacteria generally predominate except at unusual pH. It is believed that the development of protozoa is closely tied to the bacterial population on which they feed. However, Brink (*Intl. Rev. Gas. Hydr.* 52, 1, 1967) questions the importance of bacteria as food sources for protozoa and suggests that ciliates feed mainly on dead organic matter. Higher forms of life, such as worms and insects are considered to be secondary feeders in that they derive their nutrients from the degradation of other microorganisms. The thickness of the biological slime layer varies from a few microns to 0.5 cm; the steady state thickness depends on hydraulic load and availability of nutrients. Bacteria predominate at the liquid interface where there is direct access to soluble and colloidal nutrients. Fungi and filamentous forms predominate in the inner layers next to the solid support.

During the last few years a number of investigators have studies the kinetics of biological surfaces under more controlled conditions in order to obtain more detailed descriptions of the effects of operational variables and to allow making more precise measurements of the effective slime thickness. Sanders (*Air and Water Poll.*, Int. J., *10*, 235) studied the attachment and formation of slime layers on submerged surfaces in a flow system using nutrient broth medium. He reports a value of 10 microns (10^{-3} cm) for the average thickness of the first attaching layer of microorganisms. Thickness increases with time, but a steady state rate of oxygen utilization was observed after the slime thickness reached a depth of approximately 20 microns. The limiting thickness (just prior to complete break away) varied from 80 to 400 microns. It is clear that the slime layer is not a smooth and uniform surface. Sanders reports topographical variations of the same order of magnitude as the average thickness of the slime layer. The slime is periodically torn away from its attachment (after about two weeks) and the build up process starts anew. Bulk flow velocity has the expected effects; low flow rates (0.1ft/sec.) ultimately result in thicker slime layers whereas higher flow rates (1.0ft/sec.) increase the maximum uptake rate of oxygen from 38×10^{-6} to 68×10^{-6} milligrams per minute per centimeter squared of surface.

The Stevenage Laboratory of the Institute of Sewage Purification (1956 Report) has run tests using a rotating cylindrical surface to measure the effect of slime accumulations in sewage treatment. Their data show that the rate of purification increases with increasing slime mass until an amount equivalent to a thickness of 0.012 cm. has built up. Further accumulations had no effect on the rate of uptake of nutrients. Kornegay and Andrews (*J. Wat. Poll. Control Fed.*, *40*, R400, Nov. 1968) report that the maximum effective thickness of slime in an immersed film reactor is approximately 70 microns. In a study using a flat surface slime layer, the author showed that increasing slime thickness in the range of 0.05 to 0.2 centimeters had no effect on glucose uptake rate. The measured rate of glucose uptake has been compared with uptake rates measured in well mixed reaction vessels to allow estimating the effective thickness of the slime layer. A cell size of 0.5 to 1.0 microns was assumed. The results indicate that an active layer of one to two cells could account for the observed glucose uptake rate.

Studies of a number of investigators confirm the conclusion that the biological activity responsible for substrate uptake is localized in a thin film at the interface between the slime layer and liquid film. This lends support to the idea of modeling the behavior of the slime layer in terms of a catalytically active surface akin to heterogenous catalysis in a film flow reactor.

FILM FLOW REACTOR--OPERATING PROCEDURE

A flat inclined plane, illustrated in Figure A-1 (Section A) was used as a support for the slime surface. The plate, 60 cm. long and 11 cm. wide, was covered with a wire mesh as a framework to hold the slime layer in place. Growth medium was passed over the surface in a thin film. The surface was scraped periodically with a knife edge to remove cell mass and ensure uniform slime thickness. Scraping did not affect the rate of uptake of substrate. Feed and effluent samples were tested to determine substrate removal. Samples were collected over a period of 1 - 10 minutes and filtered through a 0.45 micron millipore filter to remove active cell material. The surface was allowed to equilibrate for at least 30 minutes after each change in operating conditions. Previous experience has shown that this was more than adequate to achieve steady state provided that there is no need for acclimatization of the cells to a new substrate. The data are listed in Appendix Tables 29-34.

STARCH DEGRADATION

A slime layer was grown using a feed solution containing 100mg/l. starch as the only source of carbon, and salts. The original microorganism seed was obtained from the Minneapolis-St. Paul Sewage Treatment Plant.

Four levels of starch concentration were tested at various flow rates ranging from 1 to 75 milliliters per minute per centimeter of plane width. Starch concentration was determined colorimetrically using iodine reagent and the Beckman Carbon Analyzer was used to determine organic carbon as described in Section A.

Typical results using soluble starch are shown in Figure 32, where effluent concentration is correlated with reciprocal flow rate. Effluent concentration was found to vary inversely with flow rate. This is in accord with earlier observations using glucose which showed that the quantity of substrate removed is essentially independent of flow rate. It follows that the slope of the correlation lines is a measure of the rate of substrate uptake. Increasing feed concentration from 20 mg/l starch to 50 mg/l more than doubles the slope and the rate of starch degradation. A smaller increase was observed for the 100 mg/l liter feed, while above 100 mg/l, there was essentially no change in slope. These data are summarized in Figure 33, the rate of substrate degradation is expressed as milligrams per square centimeter of slime surface per minute and is correlated with feed substrate concentration. The measured rates of starch removal are compared with the rates of starch mass transfer calculated from the diffusion control model. A value of 0.08 cm^2/sec. was used for the molecular diffusion coefficient of starch is water at 20°C. There is close agreement up to 50 mg/l feed concentration. At higher feed concentrations, the measured rates of degradation level off and become independent of feed concentration. Starch degradation in excess of that calculated by the mass transfer model was observed occasionally at low concentrations. This is believed to be due to the counter diffusion of hydrolytic enzymes and cells from the slime surface into liquid film. Spot tests show that there was continuing degradation of starch in the receiving vessel at rates of 0.1 - 0.2 milligrams per liter per minute.

Removal of organic carbon proceeds at a slower rate than starch degradation. Nevertheless, the effect of feed concentration follows the same pattern, carbon removal increases with feed carbon concentration then levels off at high feed concentrations.

The observed pattern of starch degradation and organic carbon consumption lend support to the explanation that starch degradation is mass transfer limited at low concentration and reaction rate limited at higher concentrations. The slime layer behaves like a catalytically active surface which catalized hydrolysis of starch colloids into soluble fragments; part of the solubilized carbon is consumed while the remainder diffuses back into the liquid film.

Measured carbon uptake accounts for less than 50% of the degraded starch at feed concentrations above 50 mg/l of starch. The difference between rate of carbon uptake and starch degradation indicates that these processes occur independently and possibly at different locations on the surface. The presence of hydrolytic activity in the liquid film (without any measurable carbon removal gives further support to this explanation.

Figure 32

Starch Concentration Versus Flow Rate

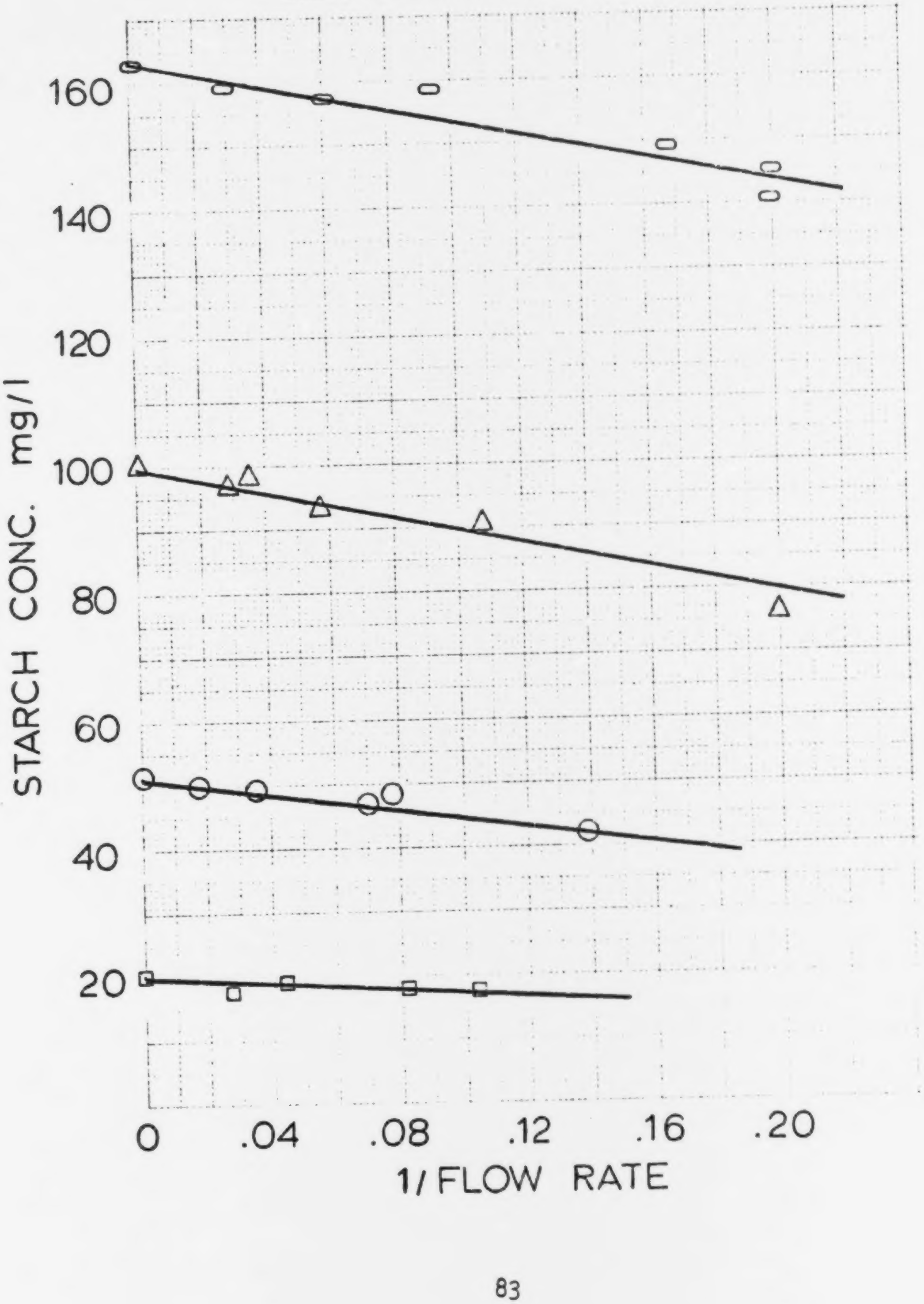
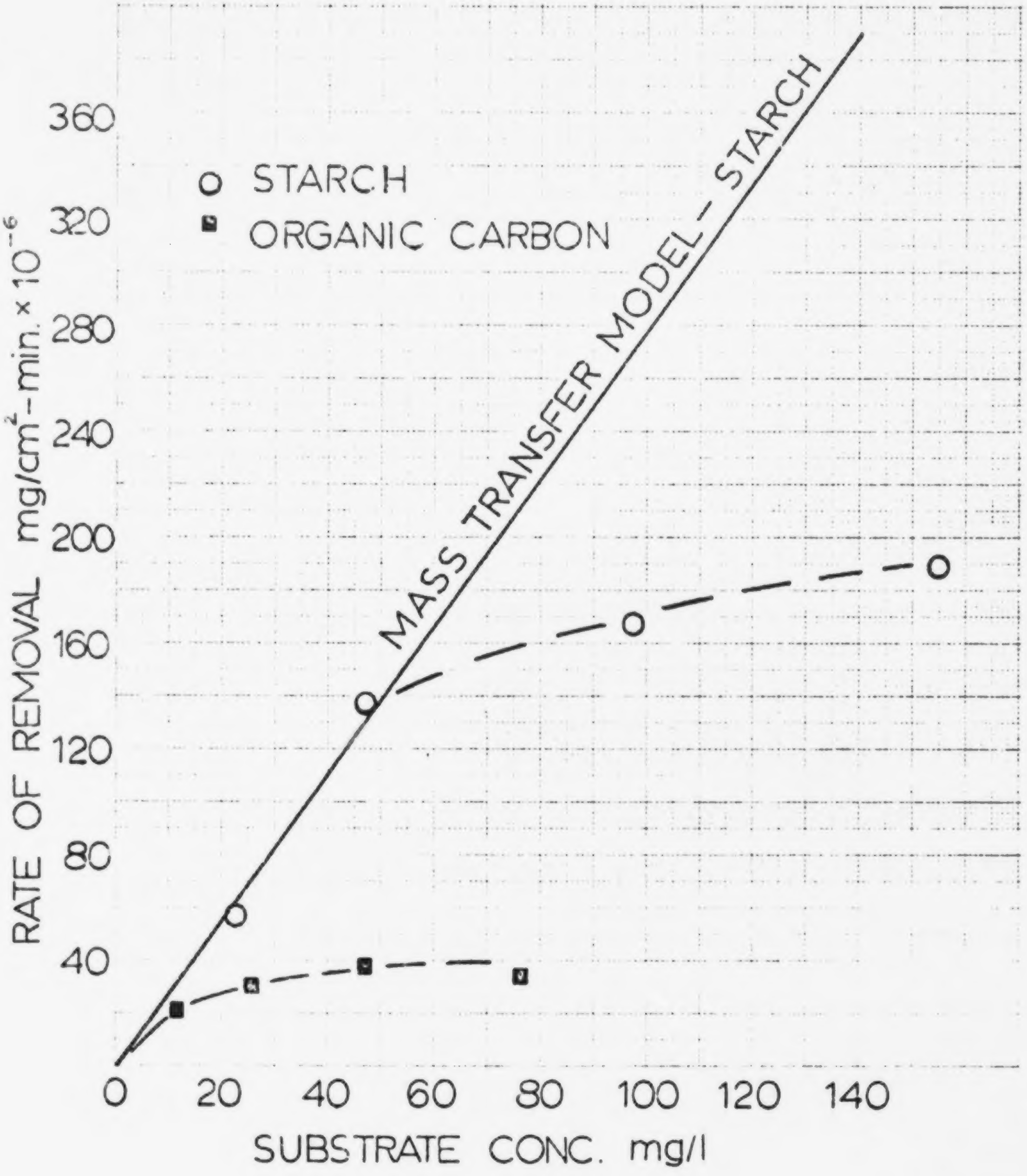


Figure 33
Starch Degradation Versus Feed Concentration



GLUCOSE-STARCH MIXTURES

Mixed feed solutions of starch and glucose were tested to determine the behavior of the slime layer in the presence of both colloidal and soluble substrate. A slime surface, which had been acclimated to feed solutions containing starch as the only source of carbon, was used. Feed and effluent samples were millipore filtered then analyzed for starch, organic carbon, and glucose. Glucose was measured colorimetrically using an enzyme reagent available from Worthington Biochemical Corporation under the trade name Gluostat. The test sequence was started with a 100mg/l starch feed followed by mixtures of starch and glucose, glucose alone and finally a mixture. Each feed was evaluated over a range of flow rates. However, the quantity of substrate removal was essentially independent of flow rate and uptake rate was expressed in terms of milligrams per centimeter squared per minute. Measured rates of removal of starch, glucose, and organic carbon are summarized below.

Feed	Feed Concentration mg/l	Carbon Concentration mg/l	Rate of Removal, mg/cm^2-min.x10^{-6}			Test
			Starch	Glucose	Carbon	
Starch	107	44	309	- - -	52	1
Starch	105	64	330	190	129	2
Glucose	53					
Starch	100	82	267	362	154	3
Glucose	99					
Glucose	100	45	- -	380	170	4
Glucose	98	63	132	320	161	5
Starch	48					

Addition of glucose had no immediate effect on the rate of starch degradation Tests #2 and #3 were carried out over a period of 4-5 hours. However, addition of glucose did increase the rate of organic carbon uptake by a factor of 2-3. Glucose uptake was nearly doubled by using a feed concentration of 100 mg/l compared to 50 mg/l in Test #2. However, the rate of glucose and carbon uptake remained essentially constant when starch was deleted during Test #4. At the end of Test #4 (about 3 hours), starch was reintroduced at 50 mg/l concentration. Starch degradation was observed, but the rate was 1/3 of that observed earlier. The reduction is larger than expected for this change in feed concentration and it seems likely that some of the enzyme activity was lost during Test #4 when growth was fueled by glucose alone.

Comparison of the rates of carbon uptake versus glucose uptake shows that glucose is almost completely consumed. By contrast, only a fraction of the degraded starch is consumed, less than 40%. These observations are consistent with the concept of a surface which has catalytic activity for both hydrolysis and carbon uptake and where the active sites are independent and physically separated. The marked increase in carbon uptake observed when glucose was added along with starch clearly shows that the carbon consuming activity was not fully utilized when starch was used. It seems plausible that carbon uptake is limited by the transport of the products of starch hydrolysis to the active carbon consuming sites. Such transport would have to be by molecular diffusion, which would account for the low rate of carbon uptake as well as the "loss" of hydrolysis products into the main liquid film.

AMINO ACID AND PROTEINS.

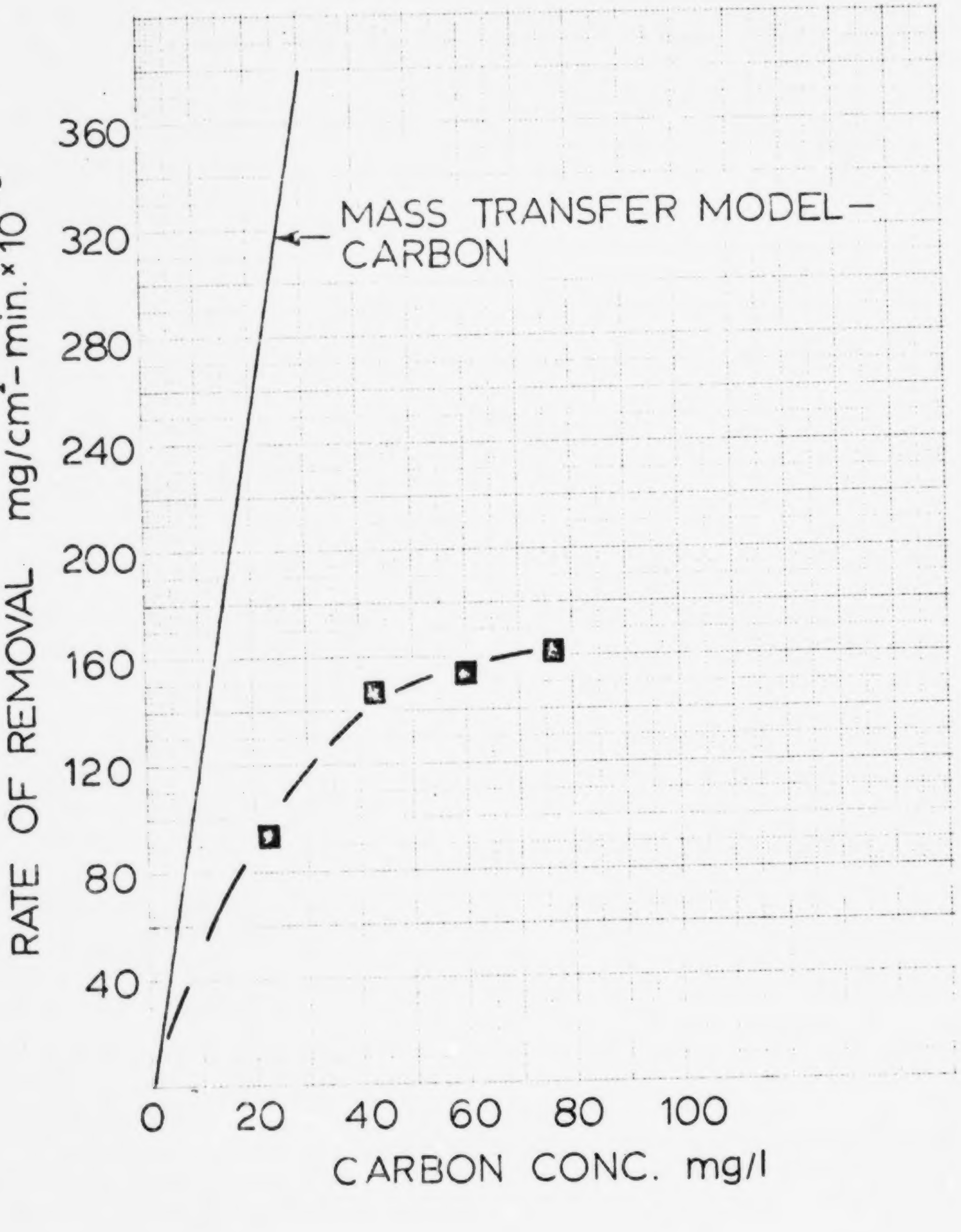
Experimental work using glutamic acid, glycine, peptone, and mixtures was designed to establish the difference in rates of uptake of soluble and colloidal substrates. Bacto peptone and bacto agar (Difco) were used as a source of peptones. Amino acids were measured with ninhydrin as described in Section 4. This test is both sensitive and reproducible but interpretation of the results is difficult because ammonium ions also react with the reagent. Protein was measured using Folin Phenol Reagent. The reagent reacts with reducing groups in proteins to give misleading results with partially hydrolized proteins. Organic carbon was measured to determine complete uptake.

In the first test, a glutamic acid feed solution (100 mg/l) plus salts and buffer was used to grow an acclimated slime layer using seed from the Minneapolis-St. Paul Sewage Treatment Plant. Four feed concentration levels of glutamic acid were tested at various flow rates. As with the starch tests, the quantity of glutamic acid removed was found to be independent of flow rates and the rates have been expressed in terms of mg/cm^2-min. Carbon rate data are shown in Figure 34 as a function of feed carbon concentration. Calculated rates of carbon transfer using the diffusion control model are shown for comparison. A diffusion coefficient of 0.63×10^{-5} cm^2/sec. was used for glutamic acid in water at 20°C and a carbon content of 40.8 wt.% was assumed for glutamic acid. Measured rates of carbon uptake increase with increasing feed concentration but level off at concentrations about 40 mg/l. Uptake of carbon is many fold less than that calculated from mass transfer considerations. Nevertheless, the initial effect of feed concentration suggests that mass transfer is a limiting factor at low concentrations.

The corresponding rates of disappearance of glutamic acid (measured with ninhydrin) are nearly independent of feed concentration. It appears that this is due to the interference of ammonium ions in the test and does not necessarily reflect the kinetics of the reactions.

Figure 34

Glutamic Acid-Carbon Removal



When a glutamic acid acclimated slime layer was exposed to a solution containing glycine as the only carbon source, there was essentially no uptake of glycine and no uptake of carbon. Furthermore, when mixtures of glycine and glutamate were used, the uptake rate was essentially that of glutamic acid. It appears that the microorganism population was unable to metabolize glycine. When peptone was substituted for glutamic acid, degradation of peptone and consumption of carbon was observed as shown below.

Feed	Concentration, mg/l feed	 carbon	Substrate Removal, mg/cm^2-min x 10^{-6} carbon	 Protein
Glutamic acid	110	46	213	- -
Peptone	200	91	120	466
Mixture*	- -	69	213	80

*50-50 mixture

Carbon removal for the peptone feed was less than that observed with glutamic acid. Mixed feeds (glutamic + peptone) showed higher carbon uptake than peptone alone. However, the slime layer had surprisingly high activity for removal of peptone as measured with Folin Phenol reagent. The measured rate of protein disappearance (460 mg/cm^2-min.) is if anything somewhat larger than that predicted from mass transfer considerations (340 mg/cm^2 min.), using a diffusion coefficient of 0.056 x 10^{-5} cm^2/sec for proteins in water at 24°C. This high proteolitic activity was not observed in all cases. It appears that under certain (as yet undefined conditions) a glutamic acid acclimated slime layer may have very little initial proteolitic activity.

Slime surface acclimated to protein substrate as its only carbon source is capable of shifting to the use of glutamic acid or mixtures of glutamic and protein without any reduction in organic carbon uptake. If anything, there appears to be a slight rate enhancement with glutamic acid as shown in the tabulation below.

	Concentration, mg/l feed	 carbon	Substrate Removal, mg/cm^2-min x 10^{-6} carbon	 protein
Protein	128	53	174	373
Glutamic acid	97	49	200	- -
Mixture	- -	47	160	173

The observed disappearance of protein is in excess of that calculated from mass trnasfer considerations using a diffusion coefficient of 0.056 x 10^{-5} cm^2/sec. Carbon removal is essentially equivalent to protein removal and indicates that mostly protein hydrolisates are consumed as they are formed, and only small amounts diffuse back into the liquid film. When peptone feed concentration was cut in half, the rate of disappearance was reduced proportionately. This effect of feed concentration is indicative of a mass transfer limitation.

In comparing the kinetics of removal of glutamic acid and protein, it is interesting to note that glutamic acid shows evidence of mass transfer limitation even though the diffusion coefficient is some 10 fold larger than that of proteins. Glutamic acid removal is less than 30% of the maximum calculated from mass transfer. Whereas, observed protein degradation rates are of the same magnitude as calculated from mass transfer considerations. This suggests that uptake of glutamic acid is limited by an additional resistance, either in terms of mass transfer, adsorption, or reaction.

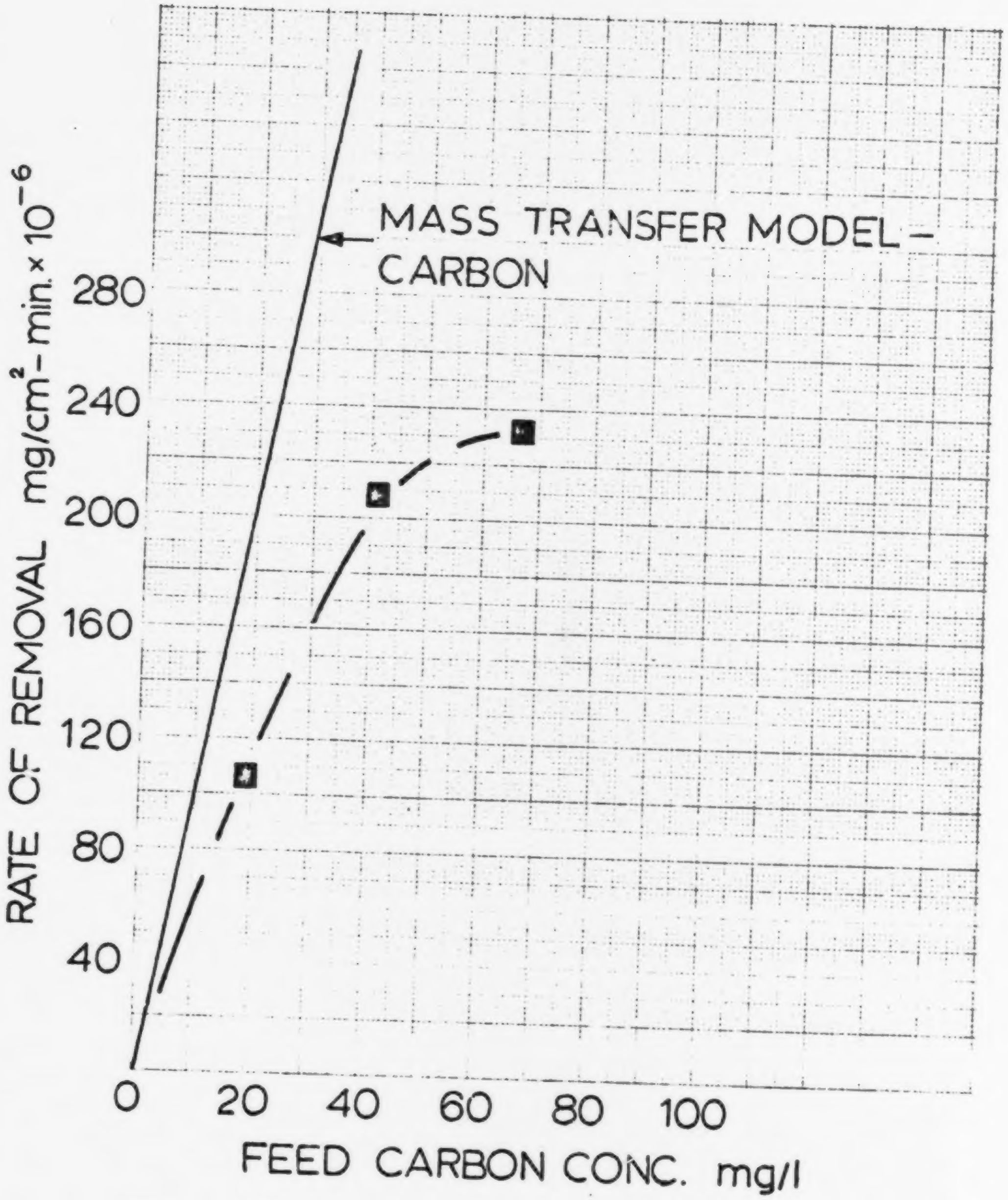
LAURIC ACID

High molecular weight fatty acids are very sparingly soluble in water. The use of alkali salts (soaps) allows forming suspensions at higher concentrations; however, these suspensions are unstable. Flocculation and settling as well as adsorption on filters makes it difficult to work with such suspensions. In order to minimize these problems and yet work with a reasonably high molecular weight fatty acid, lauric acid was used to study the kinetics of fats. As an example, lauric acid solutions could be filtered through millipore filters without substantial loss of fatty acid. However, palmitic and stearic acid could not be handled in this manner.

Biological uptake of lauric acid (no other carbon source) was tested at three concentration levels, 25, 50, and 100 mg/l. All other nutritional requirements were available in excess. A slime layer was acclimated to lauric acid feed over a period of several weeks. Carbon removal was used as a measure of biological uptake because chemical analyses for fatty acids are not sufficiently precise to measure small differences in concentration. Each feed was tested over a range of flow rates. The results show that uptake is essentially independent of flow rate. The rate data are summarized in Figure 35. Lauric acid shows the same trends as reported for other substrates, namely increasing rates of uptake as feed concentration is increased. It was not feasible to evaluate higher concentration levels due to the low solubility of lauric acid. However, it appears that maximum uptake rate is in the range 200-250 x 10^{-6} mg/cm^2-min. The observed rate data are indicative of mass transfer limitation in the same manner observed with glutamic acid substrate. The rate of mass transfer calculated from the diffusion control model using a diffusion coefficient of 0.43 x 10^{-5} cm^2/sec at 20°C is shown in Figure 35, the calculated values are substantially higher than the measured rates of uptake .

Figure 35

Lauric Acid-Carbon Removal



The lauric acid acclimated slime layer was also tested with a mixed feed consisting of approximately 100mg/l each of lauric acid and glutamic acid and glucose. The initial rate of organic carbon removal and glucose disappearance are summarized below.

	Substrate Concentration* mg/l	Substrate Removal mg/cm^2-min. x 10^{-6}
Total carbon	139	238
Glucose	(100)	48

*Mixed feed, glucose, glutamic acid and lauric acid, nominally 100mg/l of each.

Total carbon removal is essentially the same as observed with lauric acid alone. However, some glucose removal was observed and accounts for about 20% of the carbon uptake.

MIXED FEED ACCLIMATED SLIME SURFACE

A series of tests were made to determine the kinetics of substrate removal after slime layers had been acclimated to mixed substrates. The mixed substrate consisted of 100 mg/l each of glutamic acid and lauric acid and glucose. Rates of removals were measured at two levels of feed concentrations (nominally 50 and 100 mg/l each) and over a range of flow rates. Feed and effluent samples were analyzed for carbon, glucose, and amino acid concentration.

Data from the 100 mg/l feed mixture show substantially higher rates of substrate uptake then had been observed on slime layers acclimated to a single substrate. Data for carbon and glucose removal are shown below:

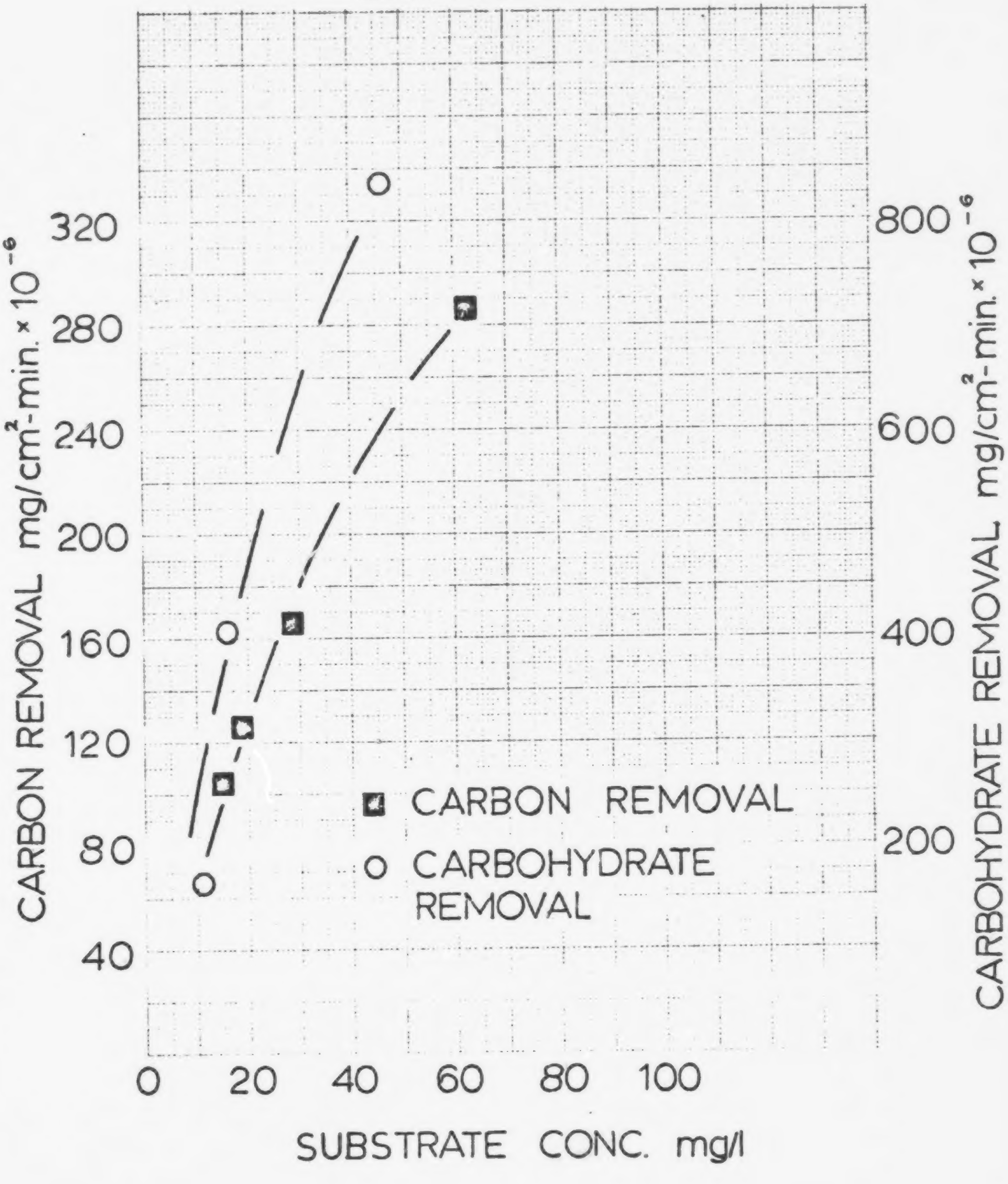
Mixed feed concentration (glucose, glutamic acid, lauric acid, mg/l of each)	100	50
	Substrate Removal, mg/cm^2-min. x 10^{-6}	
Total Carbon	410	160
Glucose	760	453

Glucose disappearance accounts for the major part of the carbon uptake. Measurements of amino acid removal are inconsistent but the data clearly point to the conclusion that rates of removal of glutamic acid are much smaller than the rates of glucose removal.

As a further test of the behavior of mixed substrates, a slime surface was acclimated to feed solutions containing skim milk solids. Skim milk solids are known to consist of high concentrations of lactose (50.5 wt.%) and protein (36.9 wt.%) plus traces of fats (0.9 wt.%) and inorganics. Growth of the slime layer was noticeably more rapid than that observed with single substrates. Rates of uptake were measured from carbon and carbohydrate disappearance using four concentration levels and a range of flow rates. Protein degradation was not determined quantitatively because the analytical techniques were inadequate. Average rates of carbon uptake and carbohydrate degradation are shown in Fig. 36 as a function of substrate concentration in the feed solution. Carbon uptake increased with increasing feed concentrations in the same manner as observed with single substrates. It appears that higher rates of removal could have been obtained by further increasing the milk solids concentration (range covered was 50-200 mg/l of dry milk solids). Carbohydrate removal accounts for most of the observed carbon uptake at all concentration levels. Nevertheless, protein degradation was noted in all tests. At low feed concentrations, protein degradation was nearly complete.

Figure 36

Skim Milk Solids Removal



DISCUSSION--SUMMARY

Substrate uptake of slime surfaces acclimated to single substrates appear to be mass transfer limited at concentrations below 100 mg/l, and reaction limited at higher concentrations. This conclusion is based on the observed effects of feed substrate concentration on uptake rate. Maximum uptake with single substrates is in the range 50 - 250 x 10^{-6} mg/cm^2-min. Highest rates were observed with lauric acid, glucose, and when mixed substrates were used. Lowest uptake rates were observed with starch feed; starch-carbon uptake was only 50 x 10^{-6} mg/cm^2-min. even though the rate of starch degradation was 2-4 fold greater. By contrast, protein removal and carbon disappearance are more nearly equivalent. Both starch and protein molecules are far too large to be absorbed directly into bacterial cells. Based on an extensive literature review, Pollock (The Bacteria, Vol. 4, Academic Press, 1962) has concluded that exoenzymes, either free or bound to the cell surface, are responsible for the hydrolytic breakdown of colloids, followed by ingestion of soluble fragments. The starch-carbon data suggest that the sites for hydrolysis and ingestion are independent and physically separated, as evidenced by the low carbon uptake rate.

Acclimation of the slime surface is necessary when new substrates are introduced; glucose is an exception. Acclimated surfaces may loose their activity for metabolizing a given substrate if the substrate is removed for any appreciable time. Some loss in activity may occur in a few hours as evidenced by the loss of hydrolytic activity for starch when glucose was substituted for approximately four hours. Acclimation of the surface to an entirely new substrate may take several days.

Acclimatization to fatty acids and to glycine are examples. It is quite likely that an entirely different surface coating (different bacterial species) is formed.

Use of mixed substrates generally results in higher rates of carbon uptake. This is particularly pronounced when carbohydrates are added. Preferential utilization of glucose in pure compound mixtures and lactose from skim milk substrates have been observed. Nevertheless, there is concurrent utilization of other substrates e.g., protein and starch degradation as well as some uptake of carbon from noncarbohydrate sources.

The results of this study provide some new insights on the behavior of biologically active slime layers in the presence of various substrates. The practical significance of this work has not been evaluated although the results do raise some questions about the practical value of certain operating practices commonly used in trickling filters. For example, the use of effluent recirculation would appear to be undesirable if BOD removal is mass transfer limited. Also the use of packing which minimizes liquid hold up seems undesirable in a mass transfer limited reactor. Further work is needed to evaluate the potential value of slime layers in biological processings and to delineate optimum operating conditions.

TABLE NO. 1

Effect of Starch Concentration on Rates of Biological Decomposition

Experiment No. 4-2-68, Batch Reactor, Well Mixed.

Preparation of Innoculum
Collected 1300 ml overflow from continuous propagator (overnight) using standard soluble starch feed solution (100 mg/l)

Preparation of Concentrates
0.8 grams of starch in 399 ml distilled water was autoclaved for 5 minutes to dissolve starch. Predetermined amounts of concentrate were added at the beginning of each test.

Sample No	Time	Starch Concentration, mg/l		Carbon Concentration mg/l
		λ=520	λ=680	
Reactor A - 300 ml innoculum + 10 ml starch concentrate				
A-0	10:00	63	65	
A-1	10:10	38	27.5	112.5
A-2	10:20	27	15.5	
A-3	10:30	18.5	10	
A-4	10:40	15	8.5	
added 30 ml of starch concentrate				
A-0	11:23	162	179	
A-1	11:33	157.5	151	
A-2	11:43	144.5	116	
A-3	11:53	127	87	
A-4	12:03	112	65	
A-5	12:23	88.5	39	
A-6	12:43	76	27	
A-7	1:03	59.5	21	
A-8	1:23	48.5	17	
A-9	1:56	38	14.5	95
Reactor B - 300 ml innoculum + 20 ml of starch concentrate				
B-0	10:02	126	128.5	
B-1	10:12	90	75.5	121
B-2	10:22	68.5	44	
B-3	10:32	56	28	
B-4	10:42	46	20	
B-5	10:52	35	15.5	
B-6	11:02	31	13	

Table No. 1 con't

Sample No	Time	Starch Concentration, mg/l $\lambda=520$	$\lambda=680$	Carbon Concentrate mg/l
added 25 ml of starch concentrate				
B-0	11:25	182	171	
B-1	11:35	149	125	
B-2	11:45	126	86	
B-3	11:55	109	61	
B-4	12:05	95.5	44	
B-5	12:25	75.5	28.5	
B-6	12:45	61	21	
B-7	1:05	51	17	
B-8	1:25	43	15.5	
B-9	1:58	34	14	97
Reactor C - 300 ml innoculum + 25 ml of starch concentrate				
C-0	10:04	153	160	
C-1	10:14	121.5	107.5	124
C-2	10:24	99	71	
C-3	10:34	80	48	
C-4	10:44	68.5	34	
C-5	10:54	59.5	26.5	
C-6	11:04	51	21	
C-7	11:14	45	17.5	
added 20 ml of starch concentrate				
C-0	11:27	170	150	
C-1	11:37	146	114	
C-2	11:47	125	82	
C-3	11:57	109.5	60	
C-4	12:07	98.5	46	
C-5	12:27	81	31	
C-6	12:47	68	24	
C-7	1:07	58	18.5	
C-8	1:27	51	18	
C-9	2:00	55	16	94

Table No. 1 con't

Sample No	Time	Starch Concentration, mg/l λ=520	Starch Concentration, mg/l λ=680	Carbon Concentrate mg/l
Reactor D - 300 ml of innoculum + 30 ml of starch concentrate				
D-0	10:06	180	183	
D-1	10:16	144.5	106	
D-2	10:26	117	88	
D-3	10:36	96.5	57.5	
D-4	10:46	83	41	
D-5	10:56	69.5	30.5	
D-6	11:06	61	24	
D-7	11:16	53	18.5	
added 10 ml of concentrate				
D-0	11:29	111	82.5	
D-1	11:39	87	47.5	
D-2	11:49	76	32.5	
D-3	11:59	67	25	
D-4	12:09	59	21	
D-5	12:29	48.5	17	
D-6	12:49	40	14	
D-7	1:09	35	13.5	
D-8				
D-9				75

Footnotes: Temperature, 23° centigrade

Innoculum was filtered (0.45 micron filter) to determine suspended solids and the "dissolved" carbon concentration.

Innoculum before addition of starch concentrate suspended solids, mg/l 35.2, 47.6
dissolved carbon, mg/l 21.0, 26.0

Yield of suspended solids in continuous propagator was determined from meansurement of dry mass and carbon concentration in filtrate and feed.

cell mass yield $\frac{\text{gram cells}}{\text{gram carbon removed}}$ 0.54, 0.795

TABLE NO. 2

Part I - Comparison of Biological Decomposition Rates of Different Starches

Experiment No. 4-30-68, Batch Reactor, Well Mixed

Preparation of Innoculum
Collected 1250 ml overflow from continuous propagator overnight (18 hours), using standard 100 mg/l soluble starch feed. Added 550 ml fresh feed (100 mg/l) at 10:00 AM and continued aeration.

Preparation of Starch Concentrate
0.8g starch added to 399 ml distilled water and autoclaved for 5 minutes to dissolve starch. Concentrate was added to innoculum to bring starch concentration to 200 mg/l.

Sample No	Time	Starch Concentration, mg/l		Carbon Concentrate mg/l
		λ=680	λ=520	
Reactor A - Evaluation of SD-5 Starch				
A-0	Innoculum	4.5	5.0	16.50
A-1	11:00	198	213	88
A-2	11:10	161	155	82
A-3	11:20	122	130	71
A-4	11:30	93	108	63
A-5	11:40	72	97	50
A-6	11:50	57	87	10
A-7	12:00	43	76	
A-8	12:20	29	59	
A-9	12:40	21	45	
Reactor B - Evaluation of SD-80 Starch				
B-0	Innoculum	4.5	6.0	16.50
B-1	11:02	192	220	96
B-2	11:12	155	192	87
B-3	11:22	128	168	83
B-4	11:32	114	152	71
B-5	11:42	99	136	62
B-6	11:52	92	127	23
B-7	12:02	78	113	
B-8	12:22	71	100	
B-9	12:42	65	80	

Table No. 2 con't.

Sample No	Time	Starch Concentration, mg/l λ=680	 λ=520	Carbon Concentrate mg/l
Reactor C - Evaluation of ES-A				
C-0	Innoculum	4.5	5.5	16.50
C-1	11:15	194	220	85
C-2	11:15	164	167	86
C-3	11:25	129	142	70.5
C-4	11:35	93	118	60
C-5	11:45	72	103	49.5
C-6	11:55	55	91	12.5
C-7	12:15	41	79	
C-8	12:25	27	62	
C-9	12:45	18	47	
Reactor D - Evaluation of Standard Soluble Starch				
D-0	Innoculum	4.5	5.0	16.50
D-1	11:07	183	212	82
D-2	11:17	134	154	79.5
D-3	11:27	93	126	68.5
D-4	11:37	64	107	52
D-5	11:47	45	93.5	41.5
D-6	11:57	35	81	21
D-7	12:07	27	71	
D-8	12:27	18	54.5	
D-9	12:47	13	42	

Footnotes: Dry weight of biomass measured on selected samples by filtering through 0.45 micron Millipore filters

	Biomass, mg/l
Innoculum	32.3, 34.5
Reactor A	105.8, 104.0
Reactor B	104.0
Reactor C	94.6

TABLE NO. 3

Part II - Comparison of Biological Decomposition Rates of Different Starches

Experiment No. 5-7-68

Preparation of innoculum and concentrates same as experiment 4-30-68.

Sample No	Time	Starch Concentration, mg/l λ=680	λ=520
Reactor A - Evaulation of PFPS Starch			
A-0	10.29	230	220
A-1	10:39	161	174
A-2	10:49	112	145
A-3	10:59	80	126
A-4	11:09	58	107
A-5	11:19	44	91
A-6	11:29	34	75
A-7	11:49	23	53
Reactor B - Evaluation of ES-G Starch			
B-0	10.31	175	164
B-1	10:41	125	129
B-2	10:51	84	105
B-3	11:01	60	90
B-4	11:11	44	79
B-5	11:21	33	67
B-6	11:31	26	56
B-7	11:51	17	39
Reactor C - Evaluation of standard soluble starch			
C-0	10.35	172	162
C-1	10:45	96	117
C-2	10:55	56	95
C-3	11:05	38	79
C-4	11:15	26	66
C-5	11:25	20	55
C-6	11:35	16	46
C-7	11:55	11	32

TABLE NO. 4

Test for "Soluble" Exo-enzyme Activity

Part I - Experiment No. 2-9-68, Batch Reactor, Well Mixed

Innoculum collected from continuous propagator using standard soluble starch feed solution of 100 mg/l. Fresh feed solution was added at the beginning of the test. Part of innoculum was filtered through 0.45 micron Millipore filter to remove cell mass.

Sample No	Time	Starch Concentration, mg/l λ=520	 λ=680
Reactor A - 100 ml innoculum + 200 ml feed solution			
A-1	2:40	61.5	62.5
A-2	2:55	36.0	28.5
A-3	3:10	23.0	13.0
A-4	3:25	10.5	7.0
Reactor B - 50 ml millipore filtered innoculum + 100 ml feed solution			
B-1	2:43	66.0	64.0
B-2	2:58	66.0	63.0
B-3	3:13	65.0	62.5
B-4	3:28	64.5	61.5
B-5	3:50	64.0	59.5
B-6	4:40	63.5	58.5

Footnotes: Temperature, 20° C.
Innoculum starch concentration, non detectable ,carbon concentration, 25 mg/l

TABLE NO.5

Part II - Experiment No. 2-10-68, Batch Reactor, Well Mixed

(Innoculum collection and test procedure same as Part I test No. 2-9-68)

Sample No	Time	Starch Concentration mg/l		Carbon Concentrate mg/l
		λ=520	λ=680	
Reactor E - 100 mg innoculum + 300 ml feed solution				
E-1	9:43	80.0		38.5
E-2	9:58	57.0	49.5	37.0
E-3	10:13	42.0	31.5	35.0
E-4	10:28	31.5	20.0	34.0
E-5	10:43	24.5	14.0	33.0
E-6	10:58	19.0	10.0	
E-7	11:13	15.5	8.0	
E-8	11:28	13.5	7.0	
E-9	11:43	11.5	6.0	32.0
E-10	11:58	8.5	5.0	30.0
E-11	12:28	4.0	3.5	
E-12	12:58	2.0	2.0	31.5
E-13	1:28	2.0	2.5	
Reactor F - 50 ml millipore filtered innoculum + 150 ml fresh feed				
F-1	9:00	80	73	
F-2	10:05	80	74	
F-3	10:20	80	74	
F-4	10:35	80	74	
F-6	11:05	79	74	

Footnotes: Temperature, 20° centigrade

Table No. 5 con't

Sample No	Time	Starch Concentration mg/l $\lambda=520$	$\lambda=680$
Reactor G - 100 ml innoculum + 300 ml fresh feed			
G-1	1:10	79.0	75.0
G-2	1:25	61.0	57.5
G-3	1:40	52.0	44.0
G-4	1:55	46.0	35.0
G-5	2:19	40.0	26.5
G-6	2:35	35.5	20.0
G-7	2:50	34.5	18.5
G-8	3:05	32.0	17.0
G-9	3:35	27.5	12.0
G-10	4:05	22.0	10.0
G-11	4:55	14.5	6.0
Reactor H - 50 ml filtered innoculum + 150 ml fresh feed			
H-1	1:44		75.5
H-3	2:21		76.0
H-5	2:55		74.5
H-6	3:10		75.5
H-7	3:50		74.0
H-8	4:15		73.5
H-9	5:00		68.0
H-10	after 17 hours		56.5

Footnotes: Temperature 21° centigrade
Innoculum starch concentration, 3 mg/l

TABLE NO. 6

Biological Decomposition of Starch-Glucose Mixtures

Experiment No. 8-29-68, Batch Reactor, Well Mixed

Preparation of Innoculum
Collected 1500 ml of overflow from continuous propagator using standard 100 mg/l soluble starch feed solution. Innoculum was collected overnight in an aerated vessel and diluted with salt solution of equal volume. Each reactor was started with 600 ml of inncoculum.

Preparation of Concentrate
One gram of starch and glucose were dissolved in 500 ml and 250 ml respectively of distilled water and autoclaved for 5 minutes. Concentrates were added to each of four reactors as shown.

Reactor	Concentrate added, ml	
	Starch	Glucose
A	30	0
B	30	15
C	60	15
D	0	15

Sample No.	Time	Starch Concentration, mg/l		Carbon	Glucose
		λ=680	λ=520	mg/l	mg/l
Reactor A					
A-0	10:36	2.0	2.5		
A-1	10:40	9.8	104		5
A-2	10:55	82.5	92		
A-3	11:10	70.5	82	53 (11:03)	1
A-4	11:25	62	76		
A-5	11:40	54	71		1
A-6	11:55	48	67		
A-7	12:10	43	65	59 (12:05)	
A-8	12:25	39.5	62		
A-9	12:40	36.5	72	49 (12:58)	1
A-10	1:11	30.5	53		
A-11	1:40	25.5	46		1
A-12	2:10	21.	38		
A-13	2:40			33 (3.04)	3
A-16	5:40	7.5		28 (3.58)	
A-17	5:30	1.5		25	
A-18	6:50			25	
A-19	7:56			25	

Table No. 6 con't

Sample No.	Time	Starch Concentration, mg/l		Carbon	Glucose
		A=680	A=520	mg/l	mg/l
Reactor B					
B-0	10:37	2.5	3.	-	0
B-1	10:42	94.5	99	-	103
B-2	10:57	79.5	85		122
B-3	11:12	66.5	77	86 (11:11)	101.5
B-4	11:27	56.5	71		102
B-5	11:42	49.	66		98
B-6	11:57	43.5	63		108
B-7	12:12	39.5	61		97
B-8	12:27	35	57		81
B-9	12:42	31.5	55		92
B-10	1:12	27		86 (1:08)	88.5
B-11	1:42	23	45		78
B-12	2:12	19.5	35		66
B-13	2:42			67 (2:30)	53
B-14	3:22			57 (3:10)	28.5
B-15	3:51			41 (4:09)	14.5
B-17	6:48	18		32 (5:30)	15.
B-18				25 (6:50)	
B-19				24 (7:56)	
Reactor C					
C-0	10:38	2.5	3		7
C-1	10:46	180	216		105
C-2	11:01	163	205	113 (11:19)	97
C-3	11:16	149	181		97
C-4	11:31	138.5	175		97
C-5	11:47	126	161		94
C-6	11:02	116	150		93.5
C-7	12:16	107.5	144	112 (12:15)	93
C-8	12:31	100	137		91.5
C-9	12:46	92	133		91
C-10	1:16	81	122	97 (1:20)	83.5
C-11	1:46	71	114		78.5
C-12	2:16	62	103	73 (2:42)	71
C-13	2:53	52.5	94		65
C-14	3:23	48	88	90 (3:21)	57.5
C-15	3:52	43.5	80		43
C-16	4:23	40	72	68 (4:24)	18.5
C-17	4:55	30.5	60	64 (6:00)	25
C-18	7:02	31.5	56	53 (7:18)	
C-18				49 (8:05)	

Table No. 6 con't

Sample No.	Time	Starch Concentrate, mg/l		Carbon	Glucose
		A=680	A=520	mg/l	mg/l
Reactor D					
D-0	10:39	2	4		0
D-1	10:48	2	3		109
D-2	11:03	2	3		109
D-3	11:18			57 (11:22)	109.5
D-4	11:33				105
D-5	11:48				105
D-6	12:03				103
D-7	12:18			55	99
D-8	12:33				97
D-9	1:18			47 (1:30)	87
D-11	1:48				76
D-12	2:18				68
D-13	2:56			40	58
D-14	3:22			34 (3:28)	49
D-15	3:56				36
D-17	4:25			25 (4:40)	4
D-18	7:02			16 (6:00)	11
D-19				14 (7:18)	
D-20				11 (8:05)	

Reactors	A	B	C
Innoculum Composition Before Test			
biomass (milipore filtered), mg/l	11	9	10
carbon content of filtrate, mg/l	12	13	11
After Test			
biomass, mg/l	36	66	94
carbon content of filtrate, mg/l	11.5	16	4

TABLE NO. 7

Effect of Glutamic Acid Concentration on Rate of Biological Decomposition

Experiment No. 12-20-68, Batch Reactor, Well Mixed.

Innoculum **Preparation**
Collected overflow from continuous propagator (110 ml/19 hours) at an average of 58 ml/hr using 100 mg/l glutamic acid feed solution. Prior to the test the innoculum was diluted with an equal volume of fresh feed solution.

Preparation of Concentrate
0.25 grams of glutamic acid was dissolved in 1000 ml of distilled water and sterilized for 5 minutes.

Sample No	Time	Concentration, mg/l	
		Carbon	Amine Nitrogen
Reactor A - 200 ml of innoculum			
A-0	11:30	26	62
A-2	12:00	26	54
A-3	12:30		60
A-4	1:00	27	60
A-5	1:30		53
A-6	2:00	25	52
A-7	2:30		51
A-8	3:00	25	58
A-9	3:30		52
A-10	4:00	22	54
A-11	6:00	21	30
A-12	7:00	20	32
A-13	10:00 A.M.	10	35
Reactor B -			
B-0	11:32	26	24
added 40 ml concentrate			
B-1	11:33	37	33
B-2	12:02	37	18
B-3	12:32		26
B-4	1:02	36	27
B-5	1:32		32
B-6	2:02	34	
B-7	2:32		29
B-8	3:02	32	26
B-9	3:32		34
B-10	4:02	30	37
B-11	6:01	25	36
B-12	7:01	27	36
B-13	10:02 A.M.	14	37

Table No. 7 con't

Sample No	Time	Concentration, mg/l Carbon	Amine Nitrogen
Reactor C -			
C-0	11:34	26	
added 80 ml concentrate			
C-1	11:34	47	112
C-2	12:04	47	
C-3	12:34		114
C-4	1:04	48	
C-5	1:34		112
C-6	2:04	47	110
C-7	2:34		110
C-8	3:04	46	108
C-9	3:34		108
C-10	4:04	41	53
C-11	6:02	36	40
C-12	7:02	36	50
C-13	10:04 A.M.	15	38
Reactor D -			
D-0	11:36	26	33
added 120 ml concentrate			
D-1	11:37	53	43
D-2	12:06	55	45
D-3	12:36		40
D-4	1:06	54	42
D-5	1:36		47
D-6	2:06	53	44
D-7	2:36		46
D-8	3:06	52	45
D-9	3:36		45
D-10	4:06	50	46
D-11	6:03	43	40
D-12	7:03	36	43
D-13	10:06 A.M.	16	43

Footnotes: Suspended solids concentrations were exceedingly low, 1-2 mg/l which is difficult to measure precisely.

TABLE NO. 8

Effect of Glutamic Acid Concentration on Rate of Biological Decomposition

Experiment No. 2-5-69, Batch Reactor, Well Mixed

Preparation of Innoculum
Collected overflow from 250 ml continuous propagator (overnight) using 100 mg/l glutamic acid feed at flow rate of 110 ml/hr. Each reactor was started with 245 ml of innoculum.

Preparation of Concentrate
0.200 grams of gultamic acid and 200 ml of distilled water were sterilized for 5 minutes. Concentrate lost some water by evaporation, final concentration was 1.03 mg/ml. Concentrate was added to each of four batch reactors at the beginning of the test.

Sample No	Time	Concentration, mg/l	
		Carbon	Amine Nitrogen
Reactor B			
B-1	10:18	6.0	2.0
	10:31	added 25 ml concentrate	
B-2	10:31	49.0	8.4
B-3	11:01	38.0	9.3
B-4	11:31	28.0	8.0
B-5	12:01	22.0	3.4
B-6	12:31	13.0	2.2
B-7	1:01	8.0	1.8
B-8	2:00	8.0	1.6
B-9	3:01		
B-10	4:01	9.0	1.6
B-11	5:11	8.0	0.8
B-12	5:49		1.6
B-13	8:15		1.5
B-14	9:40		1.8
B-15	9:30	8.0	1.9
Reactor C			
C-1	10:20	6.0	2.0
	10:33	added 40 ml concentrate	
C-2	10:33	71.0	13.2
C-3	11:02	67.0	13.7
C-4	11:32	57.0	12.7
C-5	12:02	51.0	8.5
C-6	12:32	43.0	7.4

Table No. 8 con't

Sample No	Time	Concentration, mg/l	
		Carbon	Amine Nitrogen
Reactor C con't			
C-7	1:02	24.0	4.7
C-8	2:00	9.0	2.4
C-9	3:02	9.0	1.0
C-10	4:02	10.0	1.0
C-11	5:13	7.0	1.1
C-12	5:52	11.0	1.2
C-13	8:15		1.7
C-14	9:40		2.2
C-15	9:30	11.0	2.7
Reactor D			
D-1	10:23	6.0	2.1
	10:36	added 50 ml concentrate	
D-2	10:36	85.0	16.4
D-3	11:03	80.0	17.3
D-4	11:33	71.0	15.7
D-5	12:03	61.0	11.0
D-6	12:33	52.0	11.1
D-7	1:03	34.0	6.7
D-8	2:00	12.0	0.3
D-9	3:03	13.0	1.0
D-10	4:03	14.0	0.7
D-11	5:16	13.0	1.3
D-12	5:55	13.0	1.8
D-13	8:15	16.0	2.1
D-14	9:40	16.0	2.6
D-15	9:30	16.0	3.1

Footnotes: a) The quantity of suspended material (cell mass) was determined by filtering through 0.45 micron filters and drying and weighing.

Sample filtered	Solids collected mg/l	Concentration of filtrate	
		Carbon, mg/l	Amine Nitrogen mg/l
Innoculum	30.0	10.0	0.6
Reactor B, end of test	51.0	9.0	1.8
Reactor C, end of test	82.5	12.0	2.2
Reactor D, end of test	79.0	16.0	1.3

b) Temperature was maintained at approximately 21° centigrade

TABLE NO. 9

Glycine Degradation by Glutamic Acid Acclimated Innoculum

Experiment No. 2-20-69. Batch Reactor, Well Mixed.

Innoculum Preparation
Collected overflow from continuous propagator, 2,200 ml in 15 hours, using 100 mg/l glutamic acid feed.

Preparation of Concentrate
0.404 grams of glycine was dissolved in 200 ml distilled water and sterilized for 5 minutes. Concentrate was added to innoculum at the start of each test.

Sample No	Time	Concentration, mg/l	
		Carbon	Glycine
Reactor A -			
A-1	11:35	7	7.5
	11:40	added 5.0 ml concentrate to 250 ml of innoculum	
A-2	11:40	18.5	47
A-3	12:10	23.5	50
A-4	12:40	23	52
A-5	1:10	20.5	52
A-6	1:38	27	50
A-7	2:10	25	48
A-8	3:11	23.5	37
A-9	3:55	20	32.5
A-10	5:10	23	20
A-12	11:44	19	21
Reactor B -			
B-1	11:35	7	7.5
	11:41	added 15 ml concentrate to 250 ml innoculum	
B-2	11:41	43	112
B-3	12:10	49	118
B-4	12:41	49.5	125
B-5	1:11	47.5	125
B-6	1:38	46	125
B-7	2:10	48	126
B-8	3:12	56	125
B-9	3:55	51.5	127
B-10	5:11	49	122
B-12	11:44	40	96

Table No. 9 con't

Sample No	Time	Concentration, mg/l	
		Carbon	Glycine
Reactor C -			
C-1	11:35	7	9.5
	11:42	added 25 ml concentrate to 250 ml innoculum	
C-2	11:42	66	165
C-3	12:11	79.5	187
C-4	12:42	75.5	187
C-5	1:12	74.5	187
C-6	1:39	75.5	187
C-7	2:11	83	187
C-8	3:12	100	
C-9	3:56	83	187
C-10	5:11	100	245
C-12	11:44	88	176
Reactor D -			
D-1	11:37	5	7.5
	11:43	added 35 ml concentrate to 250 ml innoculum	
D-2	11:43	93	235
D-3	12:12	98	235
D-4	12:43	99	235
D-5	1:12	97	235
D-6	1:39	100	235
D-7	2:11	95-100+	235
D-8	3:13	74	187
D-9	3:56	102	245
D-10	5:12	82	187
D-12	11:44	102	245

Footnotes: Suspended solids measured by millipore filtration

Innoculum	2.0, 8.1 mg/l
Reactor A	20.0 mg/l
Reactor C	33.7 mg/l

TABLE NO. 10

Protein and Glutamic Acid Degradation on Glutamate Acclimated Innoculum

Experiment No. 3-12-69, Batch Reactor, Well Mixed.

Innoculum Preparation
Collected overflow from continuous propagator overnight (150 ml/hr) using 100 mg/l glutamic acid feed solution. Microscopic examination showed predominance of rod shaped bacteria (length to width ratio of 3-4). Mostly single cells except for a few small clumps.

Preparation of Concentrates
0.200 grams glutamic acid + 200 ml distilled water.
0.200 grams peptone + 200 ml distilled water.
Concentrates were autoclaved for 5 minutes.

Sample No	Time	Concentration, mg/l		
		Carbon	Amino Acid	Protein
Reactor A -				
A-0	10:56	10	24	0
added 30 ml protein concentrate + 245 ml innoculum				
A-1	10:59	54	51	115
A-2	11:30	55	52	125
A-3	12:01	52	45	125
A-4	12:37	52	10	110
A-5	1:39	48	28	115
A-6	2:43	48	25	110
A-7	3:55	45	25	110
A-8	4:58	45	33	
A-9	6:23	45	25	
A-10	9:10	52	27	
A-11	10:25	44	25	
A-12	9:07	45	43	
Reactor B -				
B-0	11:01	10	18	0
added 60 ml protein concentrate + 245 ml innoculum				
B-1	11:03	90	92	210
B-2	11:31	88	97	200
B-3	12:02	88	115	225
B-4	12:37	95	13	200
B-5	1:40	84	27	205
B-6	2:43	78	44	195
B-7	3:56	75	54	185
B-8	4:59	75	51	
B-9	6:23	74	47	
B-10	9:10	52	38	
B-11	10:25	73		
B-12	9:07	65	75	

Table No. 10 con't

Sample No	Time	Concentration, mg/1 Carbon	Amino Acid	Protein
Reactor C -				
C-0	11:05	10	34	0
added 90 ml protein concentrate + 245 ml innoculum				
C-1	11:07	118	137	295
C-2	11:33	120	129	290
C-3	12:05	123	152-170	310
C-4	12:39	116	17	255
C-5	1:41	116	43	275
C-6	2:45	109	60	250
C-7	2:57	105	60	230
C-8	5:00	105	75	
C-9	6:24	105	72	
C-10	9:11	100	75	
C-11	10:26	99	55	
C-12	9:09	89	75	
Reactor D -				
D-0	11:08	10	29	
added 60 ml protein concentrate + 15 ml glutamate concentrate + 245 ml innoculum				
D-1	11:11	108	160	200
D-2	11:34	105	157	220
D-3	12:06	108	174	210
D-4	12:40	100	25	190
D-5	1:42	94	50	195
D-6	2:48	86	43	185
D-7	3:58	80	86	180
D-8	5:01	81	99	
D-9	6:24	77	90	
D-10	9:11	74	67	
D-11	10:26	74	49	
D-12	8:09	60	94	
Reactor E				
E-0	11:12	12	15	
added 60 ml protein concentrate + 30 ml gltamate concentrate +245 ml innoculum				
E-1	11:15	115		200
E-2	11:34	105	157	220
E-3	12:06	108	174	210
E-4	12:43	118	25	210
E-5	1:43	110	118	185
E-6	2:47	105	105	180
E-7	3:59	91	121	185
E-8	5:02	82		
E-9	6:25	80	129	
E-10	9:12	80	105	
E-11	10:26	80	75	
E-12	9:11	76	89	

Table No. 10 con't

Sample No	Time	Concentration, mg/l Carbon	 Amino Acid	 Protein
Reactor F -				
F-0	11:16	11	26	
added 60 ml each of protein and glutamate concentrate + 245 ml innocoulum				
F-1	11:19	144	224	175
F-2	11:37	148	247	190
F-3	12:08	156	236	195
F-4	12:43	156	26	165
F-5	1:44	140	163	175
F-6	2:47	132	115	180
F-7	4:00	124	192	175
F-8	5:03	111	217	
F-9	6:25	88	207	
F-10	9:12	78	181	
F-11	10:28	75	149	
F-12	9:11	70	115	
Reactor G - (No concentrate added)				
G-0 (22°c)	11:17	6	25	
G-2 (23°c)	11:38	11		
G-3 (24°c)	12:11	11	25	
G-4 "	12:45	16		
G-5 "	1:47	14		
G-6 "	2:47	12		
G-8	5:04	20		
G-9 "	6:26	19		
G-10 "	9:13	16		

Footnotes: Temperature of reactors is indicated by numbers in parenthesis in Reactor G data columns

Suspended solids measured by millipore filtration and drying and weighing

Innoculum	26.5 mg/l
Reactor D	7.9

TABLE NO. 11

Effect of Concentration on Biological Degradation of Peptone and Glutamic Acid using a Peptone Acclimated Culture

Experiment No. 3-18-69, Batch Reactor, Well Mixed.

Preparation of Innoculum
Collected effluent from continuous propagator overnight using 100 mg/l Bacto-Peptone feed at a flow rate of 150 ml/hr. Each reactor was started with 245 ml of innoculum.

Preparation of Concentrates
a) Dissolved 0.400 grams of Bacto-Peptone in 400 ml distilled water
b) Dissolved 0.200 grams of glutamic acid in 200 ml distilled water
Both concentrates were autoclaved for 5 minutes

Sample No	Time	Concentration, mg/l		
		Carbon	Amine Nitrogen	Peptone
Reactor A				
A-0	10:07	11	17.8	30
added 30 ml	of peptone concentrate			
A-1	10:32	51	16.5	120
A-2	11:01	50	17.5	120
A-3	11:29	48	17.5	120
A-4	11:59	48	18.0	110
A-5	12:59	40	13.9	100
A-6	1:58	28	16.4	90
A-7	2:55	24	14.8	60
A-8	3:58	16	14.8	45
A-9	4:56	23	16.2	35
A-10	8:18	16	17.6	25
A-11	10:25	15	17.5	20
Reactor B -				
B-0	10:07	11	15.1	25
added 60 ml	of peptone concentrate			
B-1	10:33	83	17.5	195
B-2	11:02	82	16.9	190
B-3	11:29	81	16.2	185
B-4	11:59	78	17.2	150
B-5	12:59	70	17.8	170
B-6	1:39	57	17.8	140
B-7	2:55	49	15.2	110
B-8	3:55	41	14.0	90
B-9	4:57	38	16.8	70
B-10	8:18	26	15.5	40
B-11	10:28	15	17.5	20

Table No. 11 con't

Sample No	Time	Concentration, mg/l		
		Carbon	Amine Nitrogen	Peptone
Reactor C -				
C-0	10:08	11	15.3	25
added 90 ml of peptone concentrate				
C-1	10:35	115	16.2	260
C-2	11:02	115	14.8	265
C-3	11:30	110	13.6	245
C-4	11:59	106	14.1	245
C-5	12:59	102	19.7	240
C-6	1:59	89	15.0	205
C-7	2:55	74	16.2	180
C-8	3:58	62	15.1	185
C-9	4:57	60	12.8	125
C-10	8:18	37	13.6	55
C-11	10:28	32	14.3	40
Reactor D -				
D-0	10:10	9	17.8	25
added 60 ml of peptone concentrate + 15 ml glutamate concentrate				
D-1	10:37	100	20.2	185
D-2	11:09	100	18.7	200
D-3	11:35	92	24.0	175
D-4	12:03	92	27.0	170
D-5	1:06	83	22.8	155
D-6	2:02	68	23.9	140
D-7	3:01	57	20.4	115
D-8	4:13	46	18.3	185
D-9	5:09	32	18.0	85
D-10	8:22	30	13.9	45
D-11	10:33	22	14.5	25
Reactor E -				
E-0	10:10	9	14.7	25
added 60 ml of peptone concentrate + 30 ml glutamate concentrate				
E-1	10:38	115	21.7	17.5
E-2	11:09	115	25.1	175
E-3	11:36	106	23.9	165
E-4	12:03	104	27.7	165
E-5	1:06	96	26.0	150
E-6	2:03	79	27.0	135
E-7	3:02	65	22.3	110
E-8	4:14	53	18.6	95
E-9	5:09	40	17.6	75
E-10	8:30	28	18.8	40
E-11	10:40	13	19.3	35

Table No. 11 con't

Sample No	Time	Concentration, mg/l Carbon	Amine Nitrogen	Peptonc
Reactor F -				
F-0	10:10	9	19.5	25
added 60 ml each of peptone and glutamate concentrate				
F-1	10:39	129	30.3	165
F-2	11:04	124	34.4	160
F-3	11:36	127	33.0	150
F-4	12:03	125	37.5	150
F-5	1:06	117	30.6	140
F-6	2:03	104	28.3	130
F-7	3:02	88	21.0	115
F-8	4:14	76	21.2	100
F-9	5:10	65	20.6	85
F-10	8:35	35	18.7	50
F-11	10:45	31	13.7	40

Footnotes: Temperature - 25°
Innoculum suspended solids concentration, 22.7, 35.2 mg/l

TABLE NO. 12

Biological Degradation of Glutamate, glycine and Peptone using Glutamate Acclimated Innoculum.

Experiment No. 3-20-69, Batch Reactor, Well Mixed.

Preparation of Innoculum
Collected effluent overnight from continuous propagator using 100 mg/l glutamic acid feed solution at approximately 130 ml/hr. Each batch test was started with 245 ml of innoculum.

Concentrate Preparation
a) 0.400 grams peptone + 400 ml distilled water
b) 0.200 grams glycine + 200 ml distilled water
c) 0.200 grams glutamic acid + 200 ml distilled water
Each concentrate was autoclaved for 5 minutes

Sample No	Time	Concentration, mg/l	
		Carbon	Amine Nitrogen
Reactor A -			
A-0	11:00	7	1.9
added 30 ml glutamic acid concentrate			
A-1	11:07	49	12.5
A-2	11:40	46	12.5
A-3	12:11	41	11.0
A-4	12:46	31	6.5
A-5	1:48	20	6.0
A-6	2:47	15	3.4
A-7	3:45	11	2.0
A-8	4:55	13	2.5
A-9	7:37	12	2.0
A-10	9:05	13	2.0
A-11	11:07	11	2.5
Reactor B -			
B-0	11:10	7	0.6
added 15 ml each of glycine and glutamic acid concentrate			
B-1	11:08	45	7.0
B-2	11:40	43	6.6
B-3	12:11	37	6.0
B-4	12:46	32	6.2
B-5	1:49	28	6.0
B-6	2:47	20	3.4
B-7	3:45	14	2.7
B-8	4:55	18	2.4
B-9	7:38	17	1.8
B-10	9:05	13	2.0
B-11	11:07	13	1.6

Table No. 12 con't

Sample No	Time	Concentration, mg/l	
		Carbon	Amine Nitrogen
Reactor C -			
C-0	11:00	7	0.6
added	30 ml glycine concentrate		
C-1	11:10	41	9.6
C-2	11:41	43	9.5
C-3	12:12	47	
C-4	12:46	44	9.5
C-5	1:49	50	11.6
C-6	2:47	43	9.3
C-7	3:45	42	10.0
C-8	4:55	44	9.1
C-9	7:38	43	8.9
C-10	9:05	40	8.3
C-11	11:07	38	8.7
Reactor D -			
D-0	11:00	7	0.6
added	30 ml glutamate and 30 ml glycine		
D-1	11:15	72	9.4
D-2	11:43	72	9.5
D-3	12:15	69	9.3
D-4	12:48	66	10.5
D-5	1:54	45	8.0
D-6	2:50	38	7.2
D-7	3:50	32	5.5
D-8	5:00	29	3.3
D-9	7:40	23	2.0
D-10	8:08	22	2.5
D-11	11:10	21	1.7
Reactor E -			
E-0	11:00	7	0.4
added	30 ml each of glycine, glutamate and peptone		
E-1	11:16	102	9.3
E-2	11:44	99	7.3
E-3	12:15	95	7.5
E-4	12:48	91	9.0
E-5	1:55	74	8.0
E-6	2:51	70	6.5
E-7	3:50	61	6.0
E-8	5:00	58	4.7
E-9	7:41	49	2.5
E-10	9:08	49	2.8
E-11	7:30	46	2.0

Table No. 12 con't

Sample No	Time	Concentration, mg/l	
		Carbon	Amine Nitrogen
Reactor F -			
F-0	11:00	12	0.4
added	30 ml each of glycine and glutamate and 90 ml peptone		
F-1	11:18	156	9.5
F-2	11:44	156	8.7
F-3	12:16	156	9.7
F-4	12:48	152	8.7
F-5	1:55	143	10.4
F-6	2:51	132	5.0
F-7	3:51	121	8.8
F-8	5:00	106	6.3
F-9	7:42	97	4.7
F-10	9:08	98	3.0
F-11	11:10	93	2.5

Footnotes: Innoculum suspended solids concentration, 23.6, 23.2 mg/l

Suspended solids at end of batch test

Reactor B	12.5 mg/l
Reactor C	14.2 mg/l
Reactor D	36.0 mg/l
Reactor E	43.0 mg/l
Reactor F	11.9 mg/l

TABLE NO. 13

Determination of Carbon Residues from Glutamic Acid Metabolism

Experiment No 6-19-69, Batch Reactor, Well Mixed

Preparation of Innoculum
Collected effluent from continuous propagator overnight (125 ml/hr). Propagator feed solution contained 100 mg/l glutamic acid. About one half of the innoculum material was filtered through a 0.45 micron Millipore filter.

Concentrate Preparation
0.400 grams of glutamic acid was dissolved in 400 ml of distilled water and autoclaved for 5 minutes.

Sample No	Time	Concentrate, mg/l	
		Carbon	Glutamic Acid
Reactor A - 200 ml of innoculum, no concentrate added			
A-1	11:35	4	13
A-3	12:53	9	13
A-4	2:26	4	8
A-5	4:40	7	7
A-6	7:35	5	7
A-7	11:17	6	8
A-8	8:07	8	10
Reactor B - 200 ml of innoculum + 20 ml of concentrate			
B-1	11:35	4	11
B-2	11:47	added concentrate	104
B-3	12:53	38	100
B-4	2:26	8	25
B-5	4:40	7	13
B-6	7:35	5	10
B-7	11:17	7	8
B-8	8:20	5	10
Reactor C - 200 ml of innoculum + 40 ml concentrate			
C-1	11:35	5	4
	11:48	added concentrate	
C-2	11:52	68	158
C-3	12:54	73	200
C-4	2:27	43	116
C-5	4:40	12	38
C-6	7:30	8	30
C-7	11:17	10	25
C-8	8:10	12	8

Table No. 13 con't

Sample No	Time	Concentrate, mg/l Carbon	Glutamic Acid
Reactor D - 50 ml each of raw innoculum and filtered innoculum			
D-1	11:35	4	3
D-3	12:55	4	5
D-4	3:00	2	2
D-5	4:40	4	1
D-6	7:28	3	2
D-7	11:17	5	15
D-8	8:12	4	5
Reactor E - 50 ml each of raw and filtered innoculum + 10 ml of concentrate			
E-1	11:40	3	4
	11:50	added concentrate	
E-2	11:54	40	67
E-3	12:59	33	108
E-4	2:31	20	60
E-5	4:45	6	13
E-6	7:26	5	15
E-7	11:17	5	4
E-8	8:14	5	3
Reactor F - 20 ml raw innoculum + 80 ml filtered innoculum + 10 ml of concentrate			
F-1	11:40	2	8
	11:52	added concentrate	
F-2	11:56	39	78
F-3	1:00	36	108
F-4	2:36	33	90
F-5	4:45	16	43
F-6	7:25	5	15
F-7	11:17	5	7
F-8	8:15	4	3

Table No. 13 con't

Sample No	Time	Concentrate, mg/l Carbon	Glutamic Acid
G	29 ml raw innoculum + 80 ml filtered innoculum		
G-1	11:40	3	
G-3	1:00	14	
G-4	2:32	2	2
G-5	4:45	4	1
G-6	7:24	3	2
G-7	11:17	3	2
G-8	8:17	2	0

Footnotes: All reactors were filtered at the end of the experiment

Reactor	Concentrate, mg/l carbon	suspended solids
A	6	21
B	7	37.4
C	11	40
D	6	8
E	6	24
F	6	26
G	4	N.D.

TABLE NO. 14

Effect of Milk Solids Concentration on Rate of Decomposition

Experiment No. 8-12-69, Batch Reactor, Well Mixed

Preparation of Innoculum
Continuous propagator was operated on 100 mg/l milk solids feed solution at 148 ml/hr. Innoculum was collected overnight.

Concentrate Preparation
0.400 grams of milk solids were dissolved in 400 ml distilled water and autoclaved.

Batch Tests
Four batch tests were set up using different amounts of concentrate. Samples were withdrawn periodically and analyzed for total carbon, carbohydrate, and protein. Effluent was collected overnight.

Batch Reactor	Innoculum	Concentrate
A	>300 ml	0 ml
B	300 ml	30 ml
C	300 ml	60 ml
D	300 ml	100 ml

Sample No	Time	Δt Minutes	Concentrate, mg/l Carbon	Carbohydrate	Protein
A-1	9:20		14	6	13
B-1	9:20		12	5	8
C-1	9:20		13	5	5
D-1	9:20		13		8
A-2					
B-2	9:27	7	33	46	18
C-2	9:29	9	49	65	30
D-2	9:30	10	73	78	65
A-3	10:30	70	13	7	10
B-3	10:31	71	40	48	45
C-3	10:32	72	61	65	66
D-3	10:33	73	77	78	72
A-4	11:25	125	12	1	15
B-4	11:25	125	38	43	25
C-4	11:24	124	59	63	66
D-4	11:26	126	75	88	62
A-5	12:28	188	16	8	8
B-5	12:29	189	33	41	32
C-5	12:25	185	55	62	61
D-5	12:29	189	71	77	32
A-6	1:34	254	13	5	8
B-6	1:37	257	29	41	25
C-6	1:33	253	52	63	48
D-6	1:33	253	62	75	40

Table No.14 con't

Sample No	Time	Δt Minutes	Concentrate, mg/l		
			Carbon	Carbohydrate	Protein
A-7	3:21	361	13	4	10
B-7	3:27	367	18	8	20
C-7	3:25	365	38	45	32
D-7	3:26	366	55	70	35
A-8	9:20	720	14	6	8
B-8	9:28	728	13	8	18
C-8	9:44	744	18	6	25
D-8	9:31	731	14	38	20
A-9	8:10	1370	14	10	10
B-9	8:19	1379	17	6	8
C-9	8:14	1374	18	13	18
D-9	8:16	1376	14	12	13

TABLE NO. 15

Biological Degradation of Milk Solids with Different Amounts of Biologically Active Solids

Experiment No. 8-18-69, Test #17

Three continuous propagators using milk solids feed solutions were set up. CP #1 ran on a 100 mg/l milk solid solution, CP #2 on a 300 mg/l solution and CP #2 on a 500 mg/l solution. They were allowed to run overnight then 100 ml aliquoto from each was filtered on 0.45 millipore filter pads to remove bacteria.

Two batch reactors were set up with innoculum taken from each continuous propagator

Batch Reactor	Innoculum	
	Raw	Filtered
A	200 ml CP#1	0 ml
B	20 ml CP#1	100 ml CP#1
C	200 ml CP#2	0 ml
D	20 ml CP#2	100 ml CP#2
E	200 ml CP#3	0 ml
F	20 ml CP#3	100 ml CP#3

Sample No	Time	Δt	Concentration, mg/l	
		Minutes	Carbon	Carbohydrate
A-1	10:11	0	28	27
B-1	10:20	9	23	11
C-1	10:11	0	72	81
D-1	10:21	10	72	80
E-1	10:24	13	96	86
F-1	10:24	13	90	87
A-2	12:10	119	28	21
B-2	12:11	120	17	5
C-2	12:13	122	72	78
D-2	12:12	121	72	80
E-2			96	86
F-2	12:16	125	85	86
A-3	2:10	239	21	13
B-3	2:11	240	17	4
C-3	2:15	244	64	76
D-3	2:13	242	70	79
E-3	2:18	247	92	81
F-3	2:19	248	84	86

Table No.15 con't

Sample No	Time	Δt Minutes	Concentration, mg/l Carbon	Carbohydrate
A-4	4:09	358	16	6
B-4	4:11	360	16	5
C-4	4:15	364	63	
D-4	4:12	361	67	77
E-4	4:25	374	73	70
F-4	4:17	366	79	86
A-5	9:07	1376	16	4
B-5	9:09	1378	17	7
C-5	9:11	1380	33	36
D-5	9:13	1382	46	56
E-5	9:24	1393	27	18
F-5	9:25	1394	53	67
A-6	10:01	2870	16	5
B-6	9:55	2864	17	7
C-6	9:43	2852	21	13
D-6	9:41	2850	29	33
E-6	9:33	2842	24	22
F-6	9:35	2845	28	27

TABLE NO. 16

Effect of Substrate Concentration for Glycine Acclimated Innoculum

Experiment No. 9-22-69, Test #19, Barch Reactor, Well Mixed

Innoculum and Concentrate Preparation

Effluent from a continuous propagator using 100 mg/l glycene feed solution was used. This material was used as innoculum for 5 batch tests. Concentrate of glycine (0.200 grams in 200 ml distilled water) and glutamic acid (0.200 grams in 200 ml distilled water) were prepared and autoclaved

Batch Reactor	Innoculum	Concentrate Additions
A	200 ml	25 ml of glycine
B	200 ml	50 ml of glycine
C	200 ml	50 ml of glutamic acid
D	200 ml	25 ml of each
E	200 ml	50 ml of each

Sample No	Time	St Minutes	Concentration, mg/l		
			Carbon	Ammonia Nitrogen	Amino Acid (as glycine)
A-1	10:10	0	27		72
B-1	10:10	0	27		93
C-1	10:12	2	27		76
D-1	10:14	4	28		75
E-1	10:17	7	27		77
(Concentrate was added prior to second sample)					
A-2	10:21	11	58	7.6	169
B-2	10:23	13	82	5.8	
C-2	10:23	13	92	12.7	152
D-2	10:27	17	89	4.7	177
E-2	10:28	18	134	12.0	
A-3	11:30	80	58		167
B-3	11:31	81	84		
C-3	11:33	[illegible]	95		148
D-3	11:35	85	88		176
E-3	11:36	86	132		
A-4	12:31	141	59		178
B-4	12:32	142	84		
C-4	12:36	146	92		153
D-4	12:37	147	89		176
E-4	12:39	149	132		
A-5	1:42	212	62	11.0	168
B-5	1:45	215	85	6.8	
C-5	1:46	216	92	12.7	154
D-5	1:49	219	89	12.9	183
E-5	1:49	219	132		

Table No.16 con't

Sample No	Time	Δt Minutes	Concentration, mg/l Carbon	Ammonia Nitrogen	Amino Acid (as glycine)
A-6	2:35	265	59		164
B-6	2:37	267	87		
C-6	2:39	269	93		147
D-6	2:41	271	89		180
E-6	2:42	272	136		
A-7	4:24	374	59		164
B-7	4:24	374	81	6.3	
C-7	4:26	376	88	12.1	148
D-7	4:27	377	80	6.6	177
E-7	4:29	379	130		
A-8	6:01	471	54	8.2	163
B-8	6:02	472	79		
C-8	6:03	473	85		147
D-8	6:06	476	79	6.3	177
E-8	6:07	477	124	12.9	
A-9	10:57	767	51	10.2	162
B-9	10:57	767	73	9.8	
C-9	11:00	770	63	17.0	129
D-9	11:00	770	50	11.4	162
E-9	11:02	772	110	14.7	
A-10	12:28	858	48	11.0	159
B-10	12:30	860	59	9.3	
C-10	12:31	861	64	17.0	128
D-10	12:32	862	49	12.9	163
E-10	12:37	867	106	16.5	
A-11	7:23		37	18.3	105
B-11	7:23	1273	51	17.4	179
C-11	7:27	1277	17	24.8	55
D-11	7:30	1280	20	12.9	74
E-11	7:35	1285	71	23.0	185
A-12	8:58	1368	30	20.0	83
B-12	8:59	1369	50	21.6	167
C-12	9:08	1374	18	27.6	48
D-12	9:08	1378	19	20.8	52
E-12	9:10	1380	63	29.1	173
A-13	11:15	1505	22	23.7	66
B-13	11:16	1506	41	27.1	142
C-13	11:17	1507	20	24.2	38
D-13	11:18	1508	18	19.5	40
E-13	11:18	1508	46	35.2	151

Table No. 16 con't

Sample No	Time	Δt Minutes	Carbon	Ammonia Nitrogen	Amino Acid (as glycine)
A-14	1:50	1660	19	24.0	48
B-14	1:50	1660	28	31.3	77
C-14	1:55	1665	20	27.0	48
D-14	1:58	1668	17	17.7	39
E-14	2:02	1672	30	37.3	102
A-15	12:47	4477	12	11.1	23
B-15	12:49	4479	16	15.2	31
C-15	12:52	4482	16	20.3	36
D-15	12:54	4484	28		
E-15	1:03	4493	20	18.9	27

TABLE NO. 17

Effect of Substrate Concentration in Degradation of Lauric Acid

Experiment No. 10-7-69, Test #20, Batch Reactor, Well Mixed

Effluent from a continuous propagator using 50 mg/l lauric acid feed was collected and used as innoculum in a series of batch tests. Different amounts of fresh feed (50 mg/l lauric acid) were added at the beginning of each batch test. The contents of the lauric acid propagator were used as innoculum. The aluric acid feed was used in lieu of concentrate.

Batch Reactor	Innoculum, ml	Fresh Feed, ml	Mixing Time
A	400	0	9:44 A.M.
B	200	200	9:44 A.M.
C	200	200	9:49 A.M.
D	40	360	9:47 A.M.

Sample No	Time	Δt Minutes	Carbon Concentration, mg/l
A-1	10:05	5	6
B-1	10:00	0	19
C-1	10:09	9	21
D-1	10:03	3	31
A-2	11:37	97	6
B-2	11:26	86	14
C-2	11:35	95	15
D-2	11:29	89	25
A-3	2:20	260	7
A-4	2:22	262	7
C-3	2:30	270	6
D-3	2:26	266	19
A-4	4:32	392	6
B-4	4:36	396	5
C-4	4:43	403	6
D-4	4:43	403	15
A-5	7:46	586	6
B-5	7:50	590	6
C-5	7:58	598	6
D-5	8:00	600	6
A-6	9:55	715	7
B-6	10:00	720	5
C-6	10:09	729	7
D-6	10:10	732	7
A-7	11:20	1320	7
B-7	11:28	1528	5
C-7	11:25	1525	5
D-7	11:26	1526	5
A-8	11:12	2952	8
B-8	11:28	2968	10
C-8	11:17	2957	8
D-8	11:32	2972	4

Footnote: Fatty acid analyses were erratic

TABLE NO. 18

Effect of Substrate Concentration in Degradation of Lauric and Palmitic Acid

Experiment No. 12-18-69, Test #21, Batch Reactor, Well Mixed

Effluent from a continuous propagator using 50 mg/l lauric acid feed was collected and used as innoculum in a series of batch tests. At the beginning of each batch test, fresh feed solution of lauric or palmitic acid were added.

Batch Reactor	Innoculum	Fresh Feed Added at Start of Tests		
		50 mg/l Lauric	100 mg/l Lauric	50 mg/l Palmitic
A	190	0	0	0
B	190	200	0	0
C	190	0	200	0
D	50	0	350	0
E	50	350	0	0
F	100	0	0	300

Sample No	Time	Δt	Carbon Concentration, mg/l
A-1	10:02		11
B-1	10:05		11
C-1	10:05		11
Fresh feed added before taking sample #2			
B-2	10:28	3	21
C-2	10:35	10	41
D-2	10:36	11	58
E-2	10:37	12	36
F-2	10:38	13	16
A-3	11:00	35	13
B-3	11:02	37	24
C-3	11:04	39	35
D-3	11:06	41	58
E-3	11:12	47	32
F-3	11:14	49	17
A-4	12:00	95	11
B-4	12:02	97	23
C-4	12:04	99	34
D-4	12:06	101	56
E-4	12:13	108	32
F-4	12:15	110	19
A-5	1:04	159	8
B-5	1:06	161	22
C-5	1:08	163	30
D-5	1:10	165	53
E-5	1:15	170	26
F-5	1:17	172	17

Table No. 18 con't

Sample No	Time	Δt	Carbon Concentration, mg/l
A-6	3:00	275	
B-6	3:02	277	19
C-6	3:04	279	25
D-6	3:06	281	48
E-6	3:12	287	26
F-6	3:14	289	16
A-7	5:00	395	
B-7	5:02	397	13
C-7	5:04	399	17
D-7	5:08	403	42
E-7	5:16	411	21
F-7	5:18	413	12
A-8	7:45	560	1
B-8	7:45	560	2
C-8	7:50	565	3
D-8	7:50	565	33
E-8	8:00	575	14
F-8	8:00	575	6
A-9	10:20	715	1
B-9	10:20	715	0
C-9	10:30	725	0
D-9	10:30	725	14
E-9	10:35	730	4
F-9	10:35	730	4
A-10	9:13	136	0
B-10	9:15	1370	0
C-10	9:17	1372	0
D-10	9:19	1374	4
E-10	9:24	1379	2
F-10	9:28	1383	2
A-11	12:00	1535	1
B-11	12:02	1537	1
C-11	12:04	1539	1
D-11	12:06	1541	3
E-11	12:14	1549	2
F-11	12:16	1551	2
A-12	3:30	1745	
B-12	3:32	1747	1
C-12	3:34	1749	1
D-12	3:36	1751	2
E-12	3:50	1765	1
F-12	3:54	1769	3

Table No.18 con't

Sample No	Time	Δt	Carbon Concentration, mg/l
B-13	11:45	2960	5
C-13	11:58	2973	3
D-13	12:10	2985	2
E-13	11:52	2967	5
F-13	11:50	2965	5
Analysis of feed solutions (F = filtered, UF = unfiltered)			
50mg/l Lauric UF	10:15		35
50mg/l Lauric F	10:15		35
100mg/l Lauric UF	10:15		65
100mg/l Lauric F	10:20		65
50mg/l Palmitic UF	10:20		33
50mg/l Palmitic F	10:18		15

Footnotes: Temperature was approximately 24°c
Cell mass determination by millipore filtration

Reactor Sample	Suspended Solids Concentration, mg/l
A	4
B	18
C	22
D	48
E	32
F	16

TABLE NO. 19

Effect of Substrate Concentration on Degradation of Palmitic and Lauric Acid.

Experiment No. 12-23-69, Test #22, Batch Reactor, Well Mixed

Effluent from a continuous propagator using 50 mg/l palmitic acid feed solution was collected and used as innoculum in a series of batch tests. At the beginning of each batch test, fresh feed solutions of lauric or plamitic acid were added.

Fresh feed added at start of test

Reactor	Innoculum	Lauric Acid Feed (m/l)		Palmitic Feed
		50 mg/l	100 mg/l	50 mg/l
A	200	0	0	0
B	200	200	0	0
C	200	0	200	0
D	50	0	350	0
E	50	350	0	0
F	100	0	0	300 ml

Sample No	Time	Δt	Carbon Concentration, mg/l
A-1	10:25	1	5
B-1	10:24	0	20
C-1	10:26	2	34
D-1	10:32	8	54
E-1	10:44	20	34
F-1	10:55	31	15
A-2			
B-2	11:29	65	14
C-2	11:30	66	28
D-2	11:31	67	41
E-2	11:39	75	21
F-2	11:45	81	8
A-3	12:20	116	3
B-3	12:21	117	7
C-3	12:22	118	20
D-3	12:23	119	48
E-3	12:29	125	23
F-3	12:51	147	8
A-4	1:30	206	6
B-4	1:21	177	4
C-4	1:22	178	12
D-4	1:23	179	45
E-4	1:35	191	21
F-4	1:49	205	6

Table No. 19 con't

Sample No	Time	Δt	Carbon Concentration
A-5	3:26	302	3
B-5	3:14	290	3
C-5	3:28	304	4
D-5	3:11	287	34
E-5	3:39	315	14
F-5	3:39	315	5
A-6	4:45	381	3
B-6	4:42	378	3
C-6	4:57	393	4
D-6	4:52	388	23
E-6	5:06	402	4
F-6	5:14	410	3
A-7	8:10	586	0
B-7	8:15	591	0
C-7	8:20	596	2
D-7	8:20	596	5
E-7	8:30	606	2
F-7	8:30	606	2
A-8	9:50	686	2
B-8	9:50	686	2
C-8	9:55	691	2
D-8	9:55	691	5
E-8	10:10	706	2
F-8	10:10	706	2
A-9	9:24	1380	3
B-9	9:25	1381	3
C-9	9:26	1382	4
D-9	9:27	1383	3
E-9	9:48	1404	2
F-9	9:50	1406	3
A-10	12:00	1536	4
B-10	12:01	1537	2
C-10	12:02	1538	3
D-10	12:03	1539	2
E-10	12:24	1560	3
F-10	12:25	1561	3

Table No. 19 con't

Sample No	Time	Δt	Carbon Concentration, mg/l
B-11	2:58	1714	3
C-11	2:59	1715	3
D-11	3:00	1716	0
E-11	3:02	1718	3
F-11	3:18	1734	4

Footnotes: Temperature was approximately 25°c

Analysis of feed solution (F = filtered, UF = unfiltered)

50 mg/l Lauric Feed UF	37
50 mg/l Lauric Feed F	38
100 mg/l Lauric Feed UF	64
100 mg/l Lauric Feed F	62
50 mg/l Palmitic Feed UF	28
50 mg/l Palmitic Feed F	15

Cell Mass Determination by millipore filtration

TABLE NO. 20

Effect of Substrate Concentration on Rate of Degradation of Mixed Feed Solutions

Experiment No. 12-30-69, Test No. 23, Batch Reactor, Well Mixed

Effluent from a continuous propagator using 50 mg/l lauric acid feed solution was collected and used as innoculum in a series of batch tests. At the beginning of each test, fresh feed solutions were added as shown:

Feed Solutions	Concentration
Lauric Acid	100 mg/l
Glutamic Acid	1000 mg/l
Glucose	1000 mg/l

Reactor	Innoculum ml	Fresh Feed Solutions Added, ml Lauric Feed	 Glucose Feed	 Glutamic Feed
A	200	200	0	0
B	200	200	50	0
C	200	200	0	50
D	200	200	50	50
E	200	200	0	0

Sample	Time	Δt	Carbon Concentration mg/l	Glucose Concentration mg/l	Glutamic Concentration mg/l
A-1	9:00	0	3		
B-1	9:00	0	3	0	
C-1	9:01	1	3		108
D-1	9:01	1	4	0	81
E-1	9:02	2	6		
A-2	9:19	19	23		
B-2	9:20	20	74	72	
C-2	9:20	20	61		114
D-2	9:21	21	95	70	107
E-2					
A-3	10:00	60	26		
B-3	10:01	61	75	74	
C-3	10:02	62	63		99
D-3	10:03	63	97	71	106
E-3	10:08	68	3		
A-4	11:00	120	16		
B-4	11:01	121	68	73	
C-4	11:02	122	57		102
D-4	11:03	123	87	61	92
E-4	11:11	131	1		
A-5	12:00	180	6		
B-5	12:01	181	55	71	
C-5	12:02	182	44		
D-5	12:03	183	77	67	97
E-5	12:15	195	1		

Table No.20 con't

Sample	Time	Δt	Carbon Concentrate mg/l	Glucose Concentrate mg/l	Glutamic Concentrate mg/l
A-6	1:00	240	4		
B-6	1:01	241	47	66	
C-6	1:01	241	37		98
D-6	1:02	242	67	32	71
E-6	1:24	264	3	32	71
A-7	1:59	299	4		
B-7	2:00	300	39	45	
C-7	2:01	301	23		87
D-7	2:02	302	47	13	83
E-7	2:28	328			
A-8	4:30	450	4	0	
B-8	4:41	461	13	1	
C-8	4:36	456	7		66
D-8	4:57	477	10	1	68
E-8	4:41	461	4		
A-9	6:40	580	4		
B-9	6:41	581	6	2	
C-9	6:42	582	8		71
D-9	6:43	583	8	9	80
E-9	7:13	613	4		
A-10	9:15	735	5		
B-10	9:16	736	5	2	
C-10	9:17	737	7		88
D-10	9:18	738	6	1	65
E-10	9:48	768	4		
A-11	9:04	1444	3		
B-11	9:05	1445	4	1	
C-11	9:26	1446	6		72
D-11	9:07	1447	7	1	74
E-11	9:32	1472	5		
A-12	2:05	1745	3		
B-12	2:17	1757	2	0	
C-12	2:15	1755	5		85
D-12	2:35	1777	3	8	75
E-12	2:09	1749	2		
A-13	2:29	4649	7		
B-13	2:34	4654	10	8	
C-13	2:58	4678	10		80
D-13	3:20	4700	10	8	83

Footnotes: a) Temperature was approximately 28°c, b) Suspended solids (cell mass) was measured by filtering through a membrane filter and weighing the dry mass.

Reactor	Suspended Solids, mg/l
B	34
C	34

c) High amino acid concentration is due to presence of ammonia nitrogen

Sample	C-1	C-6	C-11	D-1	D-5	D-10
Ammonia Nitrogen, mg/l	106.5	80.5	95.0	115.5	85.0	78.5

TABLE NO. 21

Effect of Substrate Concentrations on Rate of Degradation of Mixed Feed Solutions.

Experiment No. 1-29-70, Test No. 24, Batch Reactor, Well Mixed

Effluent from a continuous propagator using 50 mg/l lauric acid feed solution was collected and used on innoculum in a series of batch tests. At the beginning of each test, fresh feed solutions were added as shown.

Feed Solution	Concentration, mg/l
Lauric Acid	100
Glutamic Acid	1000
Glucose	1000

Reactor	Innoculum (ml)	Fresh Feed Solutions Added		
		Lauric Feed	Glucose Feed	Glutamic Feed
A	200	200 ml	0 ml	0 ml
B	200	200 ml	50 ml	0 ml
C	200	200 ml	0 ml	50 ml
D	200	200 ml	50 ml	50 ml
E	200	0 ml	0 ml	0 ml

Sample	Time	Δt	Carbon Concentrate (mg/1)	Glucose Concentrate (mg/1)	Glutamic Acid Concentrate (mg/1)
A-0	10:48	0	12		
B-0	10:48	0	16	2	
C-0	10:49	1	14		12
D-0	10:49	1	14	8	20
E-0	10:54	6	14		
A-1	11:00	12	35		
B-1	11:01	13	79	78	
C-1	11:02	14	63		72
D-1	11:04	16	96	73	87
E-1					
A-2	11:55	67	37		
B-2	11:56	68	77	80	
C-2	11:57	69	59		90
D-2	11:58	70	97	79	87
E-2	12:01	73	12		
A-3	1:06	138	29		
B-3	1:07	139	70	81	
C-3	1:08	140	53		89
D-3	1:09	141	90	77	88
E-3	1:15	147	9		
A-4	2:00	192	25		
B-4	2:01	193	63	82	
C-4	2:02	194	49		97
D-4	2:03	195	83	79	92
E-4	2:08	200	6		

Table No. 21 con't

Sample	Time	Δt	Carbon Concentrate (mg/1)	Glucose Concentrate (mg/1)	Glutamic Acid Concentrate (mg/1)
A-5	3:04	256	16		
B-5	3:05	257	56	80	
C-5	3:06	258	44		74
D-5	3:07	259	79	81	55
E-5	3:12	264	5		
A-6	4:03	315	14		
B-6	4:05	317	55	79	
C-6	4:07	319	40		70
D-6	4:09	321	76	79	69
E-6	4:12	324	5		
A-7	6:25	457	11		
B-7	6:25	457	54	80	
C-7	6:30	462	24		44
D-7	6:30	462	57	73	45
E-7	6:35	467	4		
A-8	8:02	554	9		
B-8	8:03	555	51	77	
C-8	8:04	556	10		20
D-8	8:05	557	40	43	28
E-8	8:16	568	4		
A-9	9:57	669	6		
B-9	9:58	670	49	78	
C-9	9:59	671	9		21
D-9	10:00	672	23	9	26
E-9	10:08	680	4		
A-10	11:59	791	4		
B-10	12:00	792	45	70	
C-10	12:03	795	9		21
D-10	12:05	797	18	2	12
E-10	12:11	803	6		
overnight 1/29 to 1/30					
A-11	10:27	1419	5		
B-11	10:32	1424	39	26	
C-11	10:39	1431	10		25
D-11	10:54	1446	14	3	18
E-11	10:31	1423	8		
A-12	12:48	1560	5		
B-12	12:52	1564	33	21	
C-12	1:00	1572	10		25
D-12	1:07	1579	13	5	11
E-12	12:50	1562	9		

Table No. 21 con't

Sample	Time	Δt	Carbon Concentrate (mg/1)	Glucose Concentrate (mg/1)	Glutamic Acid Concentrate (mg/1)
A-13	9:45	2757	6		
B-13	9:46	2758	5	2	
C-13	9:47	2759	9		31
D-13	9:48	2760	11	0	11
E-13	10:05	2777	8		

Footnotes: a) Temperature was maintained at approximately 27°c

b) Suspended solids were measured by membrane filtration and dry weighing

Reactor	Suspended Solids, mg/1
A	6
B	34
C	26
D	58
E	4

c) Greenish tint developed in Reactor C and D after 12 hours

d) Glucose analysis by phenol sulfuric acid test

TABLE NO. 22

Effect of Substrate Concentration on Rate of Degradation of Mixed Feed Solutions

Experiment No. 2-23-70, Test No. 25, Batch Reactor, Well Mixed

Effluent from a continuous propagator using a mixed feed consisting of 100 mg/l lauric acid, 100 mg/l glutamic acid and 100 mg/l of glucose was collected and used as innoculum in a series of batch tests. At the beginning of each test, fresh feed solutions were added as shown:

Feed Solutions	Concentration, mg/l
Lauric Acid	100
Glutamic Acid	1000
Glucose	1000

Reactor	Inncoulum (ml)	Frech Feed Solutions Added		
		Lauric Feed (ml)	Glucose Feed (ml)	Glutamic Feed (ml)
A	200	0	25	0
B	200	0	0	25
C	200	200	0	0
D	200	200	25	25
E	200	0	0	0

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-0	10:48	0	44	34	
B-0	10:49	1	42		4
C-0	10:50	2	42		
D-0					
E-0	10:51	3	41		
A-1	11:49	61	76	89	
B-1	11:49	61	58		70
C-1	11:50	62	42		
D-1	11:50	62	68	67	45
E-1	11:51	63	34		
A-2	1:02	134	72	74	
B-2	1:09	141	45		
C-2	1:21	153	35		
D-2	2:26	218	35	30	6
E-2	2:28	220	22		
A-3	2:30	222	63	68	
B-3	2:34	226	20		13
C-3	3:31	283	27		
D-3	3:43	295	27	9	5
E-3	3:49	301	18		

Table No. 22 con't

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-4	4:10	322	58	43	
B-4	4:22	334	16		
C-4	4:53	365	24		
D-4	5:14	386	12	0	2
E-4	5:18	390	13		
A-5	7:24	516	45	7	
B-5	7:25	517	15		12
C-5	7:26	518	17		
D-5	7:27	519	11	0	4
E-5	8:06	558	13		
A-6	9:38	650	40	21	
B-6	9:39	651	15		14
C-6	9:40	652	11		
D-6	9:41	653	7	12	6
E-6	10:40	712	13		
A-7	9:30	1362	20	25	
B-7	9:30	1362	21		22
C-7	9:31	1363	6		
D-7	9:32	1364	11	80	4
E-7	10:15	1407	16		
A-8	12:05	1517	13	23	
B-8	12:06	1518	21		21
C-8	12:07	1519	7		
D-8	12:08	1520	8	34	6
E-8	12:46	1558	15		
A-9	2:28	1660	11	11	
B-9	2:29	1661	20		26
C-9	2:30	1662	7		
D-9	2:31	1663	10	12	12
E-9	3:18	1710	15		
A-10	9:52	2824	12	14	
B-10	9:53	2825	21		16
C-10	9:54	2826	7		
D-10	9:55	2827	11	8	19
E-10	10:24	2856	15		

Table No. 22 con't

Sample	Time	Δt	Carbon Concentrate (mg/l)	Gyucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-11	10:17	4289	15	19	
B-11	10:18	4290	20		11
C-11	10:19	4291	7		
D-11	10:20	4292	11	3	24
E-11	10:58	4330	16		

Footnotes: a) Temperature was approximately 27°c

b) Suspended solids were measured at the end of each test by filtering through a membrane filter and dry weight

Reactor	Suspended Solids Concentrate, mg/l
A	112
B	102
C	50
D	92
E	96

c) Some protozoa were detected in batch tests B, C, D, and E

TABLE NO. 23

Effect of Substrate Concentration on Rate of Degradation of Mixed Feed

Experiment No. 4-30-70, Test No. 26, Batch Reactor, Well Mixed

Effluent was collected from a continuous propagator using a mixed feed solution of 100 mg/l each of glucose, glutamic acid and lauric acid and was used as innoculum in a series of batch tests. Fresh feed solutions were added at the beginning of each batch test as shown:

Fresh Feed Solution	Concentration, mg/l
Lauric Acid	100
Glutamic Acid	1000
Glucose	1000

Reactor	Innoculum (ml)	Fresh Feed Solution Added		
		Lauric Concentrate (ml)	Glucose Concentrate (ml)	Glutamic Concentrate (ml)
A	200	200	0	0
B	200	200	50	0
C	200	200	0	50
D	200	200	50	50
E	200	0	0	0

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-0	1:08	4	39		
B-0	1:04	0	37	33.8	
C-0	1:06	2	37		5
D-0	1:08	4	37	25.0	7
E-0	1:15	11	39		
A-1	1:43	39	45		
B-1	1:44	40	72	70.0	
C-1	1:45	41	66		88
D-1	1:44	40	93	80.0	73
E-1	1:44	40	47		
A-2	4:39	175	45		
B-2	4:37	173	71	95.0	
C-2	4:45	181	43		45
D-2	4:45	181	76	80.0	40
E-2	5:00	196	33		
A-3	6:08	304	33		
B-3	6:06	302	65	70.0	
C-3	6:12	308	27		10
D-3	6:16	312	59	77.0	8
E-3	6:39	335	27		

Table No. 23 con't

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-4	7:38	394	30		
B-4	7:39	395	56	47.3	
C-4	7:40	396	14		8
D-4	7:41	397	38	56.3	10
E-4	7:42	398	24		
A-5	10:30	566	22		
B-5	10:30	566	51	81.3	
C-5	10:32	568	10		12
D-5	10:32	568	22	25.6	10
E-5	10:34	570	14		
A-6	9:10	1206			
B-6	9:11	1207	34	48.8	
C-6	9:11	1207	10		13
D-6	9:23	1219	10	15.0	5
E-6	9:23	1219	10		
A-7	4:22	1638	15		
B-7	4:23	1639	28	42.3	
C-7	4:23	1639	11		11
D-7	4:32	1648	13	22.0	8
E-7	4:32	1648	10		
A-8	1:06	2882	12		
B-8	1:20	2896	16	19.0	
C-8	12:58	2874	12		12
D-8	1:20	2896	13	20.0	13
E-8	1:11	2887	12		

TABLE NO. 24

Effect of Substrate Concentration on Rate of Degradation of Mixed Feed

Experiment No. 5-21-70, Test #27, Batch Reactor, Well Mixed

Effluent was collected from a continuous propagator using a mixed feed of 100 mg/l each of glucose, glutamic acid and lauric acid and was used as innoculum in a series of batch tests. Fresh solutions were added to at the beginning of each batch test.

Feed Solution	Concentration, mg/l
Lauric Acid	100
Glutamic Acid	1000
Glucose	1000

Reactor	Innoculum (ml)	Feed Solutions Added Lauric Feed (ml)	Glucose Feed (ml)	Glutamic Feed (ml)
A	200	0	0	25
B	200	0	25	0
C	200	200	0	0
D	200	200	25	25
E	200	0	0	0

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-0	11:59	12	11		92
B-0	11:52	5	10	6	
C-0	11:48	1	9		
D-0	11:50	3	11	4	
E-0	11:47	0	11		
A-1	12:27	40	39		141
B-1	12:30	43	30	45	
C-1	12:23	36	27		
D-1	12:22	35	48	23	
E-1	12:27	40	9		
A-2	2:44	177	11		117
B-2	2:53	186	7	12	
C-2	2:38	171	12		
D-2	2:41	174	14	3	
E-2	2:43	176	7		
A-3	6:50	423	8		160
B-3	6:55	428	7	6	
C-3	6:40	413	6		
D-3	6:42	415	8	2	
E-3	6:44	417	6		

Table No. 24 con't

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-4	8:53	546	11		135
B-4	8:57	550	10		
C-4	8:43	536	7		
D-4	8:49	542	8	4	
E-4	8:47	540	7		
A-5	11:07	680	8		140
B-5	11:08	681	10	8	
C-5	10:50	663	7		
D-5	10:50	663	8	2	
E-5	10:51	664	7		
A-6	1:33	826	10		127
B-6	1:36	829	6	0	
C-6	1:25	818	6		
D-6	1:28	821	6	2	
E-6	1:28	821	8		
A-7	2:50	1623	7		103
B-7	2:56	1629	6	0	
C-7	3:43	1616	7		
D-7	3:49	1622	8	0	
E-7	2:48	1621	5		

Footnotes: a) Sample L-1 foamed when glutamic acid was added and some foam was lost

TABLE NO. 25

Effect of Substrate Concentration on Rate of Degradation Using Mixed Feed and Excess Ammonia Nitrogen

Experiment No. 6-19-70, Test #29, Batch Reactor, Well Mixed

Effluent was collected from a continuous propagator using a mixed feed of 100 mg/l each of glucose, glutamic acid and lauric acid and was used as innoculum in a series of batch tests. Fresh solutions were added to at the beginning of each batch test.

Feed Solutions	Concentrations, mg/l
Glutamic Acid	200
Lauric Acid	200
Glucose	200
NH_4Cl	200

Reactor	Innoculum ml	Lauric Concentrate ml	Glucose Concentrate ml	Glutamic Acid Concentrate ml	NH_4Cl Concentrate ml
A	100	200	0	0	100
B	100	0	200	0	100
C	200	0	0	200	0

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-0	9:55	0	10	16	165
B-0	9:58	0	12	16	112
C-0	10:04	0	13	16	116
A-1	10:12	2	128		
B-1	10:10	0	64	51	
C-1	10:10	0	56		145
A-2	11:18	68	123		
B-2	11:20	70	60	53	
C-2	11:18	68	53		141
A-3	12:39	149	110		
B-3	12:42	152	57	37	
C-3	12:59	169	48		153
A-4	1:48	218	94		
B-4	1:55	225	44	43	
C-4	1:59	229	40		72
A-5	2:47	277	82		
B-5	2:52	282	32.5	23	
C-5	2:49	279	31		45
A-6	3:52	342	65		
B-6	4:00	350	27	7	
C-6	4:00	350	24		37

Table No. 25 con't

Sample	Time	Δt	Carbon Concentrate (mg/1)	Glucose Concentrate (mg/1)	Glutamic Acid Concentrate (mg/1)
A-7	4:48	398	56		
B-7	4:52	402	27	8	
C-7	4:52	402	20		50
A-8	8:31	621	41		
B-8	8:32	622	20	6	
C-8	8:33	623	15		71
A-9	10:33	743	38		
B-9	10:30	740	19	9	
C-9	10:37	747	12		59
A-10	12:22	852	33		
B-10	12:21	851	11	7	
C-10	12:23	853	9		77
A-11	9:47	1417	29		
B-11	9:45	1415	15	8	
C-11	9:45	1415	9		75
A-12	8:15	2045	30		
B-12	8:13	2043	10	8	
C-12	8:13	2043	7		92
A-13	6:43	3397	32		
B-13	6:43	3393	9	7	
C-13	6:43	3393	9		62

TABLE NO. 26

Effect of Substrate Concentration on Rate of Degradation Using Mixed Feed and Excess Ammonia Nitrogen

Experiment No. 7-15-70, Test No. 30, Batch Reactor, Well Mixed

Effluent was collected from a continuous propagator using a mixed feed of 100 mg/l each of glucose, glutamic acid and lauric acid and was used as innoculum in a series of batch tests. Fresh solutions were added to at the beginning of each batch test.

Feed Solutions	Concentration, mg/l
Lauric Acid	1000
Glutamic Acid	1000
Glucose	1000
Ammonium Chloride	1000

Reactor	Innoculum Volume (ml)	Lauric Concentrate Volume(ml)	Glucose Concentrate Volume(ml)	Glutamic Acid Concentrate Volume(ml)	NH_4Cl Volume (ml)
A	340	40	0	0	20
B	340	0	40	0	20
C	360	0	0	40	0
D	280	40	40	40	0

Sample	Time	Δt	Carbon Concentrate (mg/l)	Glucose Concentrate (mg/l)	Glutamic Acid Concentrate (mg/l)
A-0	11:30	0	79	30	4
B-0	11:30	0	78	55	20
C-0	11:30	0	76	33	22
D-0	11:30	0	80	75	28
A-1	12:05	35	129		
B-1	12:05	35	117	140	
C-1	12:05	35	98		78
D-1	12:10	40	160	135	87
A-2	1:09	99	120		
B-2	1:10	100	105	128	
C-2	1:11	101	94		68
D-2	1:12	102	155	139	87
A-3	2:19	169	110		
B-3	2:20	170	99	125	
C-3	2:20	170	87		51
D-3	2:17	167	154	133	56
A-4	3:17	227	134		
B-4	3:18	228	94	116	
C-4	3:19	229	77		37
D-4	3:45	245	167	116	41

Table No. 26 con't

Sample	Time	Δt	Carbon Concentrate (mg/1)	Glucose Concentrate (mg/1)	Glutamic Acid Concentrate (mg/1)
A-5	4:11	281	98		
B-5	4:13	283	83	109	
C-5	4:14	284	63		17
D-5	4:18	288	149	125	22
A-6	5:45	375	80		
B-6	5:46	376	63	88	
C-6	5:47	377	49		6
D-6	5:50	380	133	108	7
A-7	6:05	395	126		
B-7	6:08	398	114	87	
C-7	6:15	405	95		16
D-7	6:18	408	187	87	9
A-8	8:25	535	42		
B-8	8:27	537	45	47	
C-8	8:32	542	30		5
D-8	8:30	540	109	93	6
A-9	9:35	605	32		
B-9	9:36	606	39	22	
C-9	9:37	607	31		5
D-9	9:31	601	115	68	7
A-10	10:25	655	31		
B-10	10:26	656	28	10	
C-10	10:26	656	32		5
D-10	10:27	657	107	48	5
A-11	9:46	1336	24		
B-11	9:47	1337	20	2	
C-11	9:47	1337	31		8
D-11	9:50	1340	62	5	6
A-12	4:23	1733	24		
B-12	4:23	1733	20	2	
C-12	4:21	1731	34		16
D-12	4:27	1737	47	7	6
A-13	9:38	2768	21		
B-13	9:35	2765	17	4	
C-13	9:37	2767	31		18
D-13	9:50	2780	34	2	6

Footnotes: Temperature was approximately 30°c-at the end of each test the material was filtered and dried to measure suspended solids (cell mass)

Reactor	Suspended Solids, mg/1
A	118
B	84
C	50
D	152

TABLE NO. 27

Effect of Substrate Type on Rate of Degradation Using Mixed Liquor Activated Sludge from Minneapolis-St. Paul Sewage Treatment Plant

Experiment No. 7-23-70, Test #31, Batch Reactor, Well Mixed

Mixed liquor was obtained from the treatment plant at about 9:00 A.M. Aliquots were used as innoculum of six batch reactors. Feed solutions were added as shown.

Feed Solutions	Concentration, mg/1
Glucose	1000
Starch	1000
Glutamic Acid	1000
Peptone	1000
Lauric Acid	100

Reactor	Innoculum Used	Feed Solutions Added				
		Glucose (ml)	Starch (ml)	Glutamic Acid (ml)	Peptone (ml)	Lauric Acid (ml)
A	200	50	0	0	0	0
B	200	0	50	0	0	0
C	200	0	0	50	0	0
D	200	0	0	0	50	0
E	200	0	0	0	0	200
F	200	25	0	25	0	50

Sample No	Time	At Min	Carbon Total mg/1	Inorganic Carbon mg/1	Protein mg/1	Glucose mg/1	Glutamic Acid mg/1	Carbohydrate mg/1	Ammonia mg/1	Starch mg/1
AI			89	26		6		7		
BI			93	27				7		3
CI			96	27			5		7.0	
DI			93	27			7		6.6	
EI			93	27						
A0	1:25	0	132	21		128		152		
B0	1:25	0	135	26				18		
C0	1:25	0	132	21			150		4.9	
D0	1:25	0	159	26	250		59		9.0	
E0	1:43	18	89	30						
F0	1:40	15	129	27		54	74		9.5	
A1	2:45	80	111	21		95		111		
B1	2:45	80	119	19				146		0
C1	2:45	80	114	24			125		9.3	
D1	2:45	80	119	23	190		43		11.1	
E1	2:57	92	34	31						
F1	2:53	88	94	25			28		2.3	

Table No. 27 con't

Sample No	Time	Δt Min	Carbon Total mg/l	Inorganic Carbon mg/l	Protein mg/l	Glucose mg/l	Glutamic Acid mg/l	Carbohydrate mg/l	Ammonia mg/l	Starch mg/l
A2	3:45	140	108	20		78		98		
B2	3:45	140	89	20				130		0
C2	3:45	140	105	25			101			
D2	3:45	140	111	25	270		30			
E2	3:55	150	94	30						
F2	3:57	152	81	25		4	3			
A3	4:55	210	94	20		57		87		
B3	4:53	208	108	19				132		0
C3	4:56	211	86	22			58		14.3	
D3	4:57	212	103	22	255		24		12.9	
E3	5:03	218	94	30						
F3	5:02	217	81	25		7	3		2.7	
A4	7:15	350	81	19		21		47		0
B4	7:15	350	111	20				120		
C4	7:20	355	81	23			22			
D4	7:23	358	100	23	235		25			
E4	7:25	360	100	32						
F4	7:25	360	78	24			2			
A5	9:04	459	75	19		7		20		
B5	9:04	459	108	20				122		0
C5	9:06	461	81	21			17		11.3	
D5	9:06	461	97	21	25		20		10.5	
E5	9:07	462	105	32						
F5	9:07	462	80	23		6	3		3.0	
A6	10:45	560	75	19		4		21		
B6	10:46	561	105	20				112		0
C6	10:45	560	75	21			18			
D6	10:54	569	86	21	215		16			
E6	10:50	565	103	32						
F6	10:49	564	73	24		6	2			
A7	9:23	1198	75	21		5		15	-	0
B7	9:23	1198	105	21				124		
C7	9:26	1201	75	24			15			
D7	9:31	1206	86	21	205		16			
E7	9:31	1206	100	34						
F7	9:32	1207	75	25		6	7			
A8	4:03	1598	75	20		5		19		0
B8	4:03	1598	100	21				118		
C8	4:03	1598	81	22			11			
D8	4:10	1605	86	21	190		18			
E8	4:12	1607	84	26						
F8	4:12	1607		34		7	2			

Footnotes: Temperature was approximately 28° C
Volatile suspended solids of innoculum was 670 mg/l
Total suspended solids of innoculum was 837 mg/l

TABLE NO. 28

Effect of Substrate Type on Rate of Degradation Using Mixed Liquor Activated Sludge from Minneapolis-St. Paul Sewage Treatment Plant

Experiment No. 8-11-70, Test #32, Batch Reactor, Well Mixed

Mixed liquor was obtained from the treatment plant at 10:00 A.M. just prior to start of the test. Aliquots were used as innoculum for each batch reactor. Feed solutions were added at the beginning of each test as shown. A second addition of fresh feed solution was also made after sample number five (after about 7 hours)

Feed Solutions	Concentration, mg/1
Glucose	1000
Starch	1000
Glutamic Acid	1000
Peptone	1000
Lauric Acid	100
Ammonium Chloride	1600

Reactor	Innoculum	Feed Solutions Added, ml					
	ml	NH_4Cl	Glucose	Starch	Glutamic Acid	Peptone	Lauric Acid
A	200	5	40	0	0	0	0
B	200	5	0	40	0	0	0
C	200	0	0	0	40	0	0
D	200	0	0	0	0	40	0
E	200	5	0	0	0	0	160
F	200	5	20	0	20	0	40
G	200	5	0	20	0	20	40

Sample No	Time	Δt	Inorganic Carbon mg/1	Total Carbon mg/1	Glucose mg/1	Protein mg/1	Glutamic Acid mg/1	Starch		Ammonia mg/1
								λ=520	λ=680	
A-I	12:40	0	27	90	0					
B-I	12:40	0	26	88				2	0	
C-I	12:40	0	27	91			0			
D-I	12:45	5	26	91		73	2			
E-I	12:45	5	26	90						
F-I	12:45	5	27	91	20		3			
G-I	12:45	5	26	88		110	7	0	0	
A-0	1:25	45	22	137	164					
B-0	1:25	45	22	140				64	24	
C-0	1:27	47	16	120			82			
D-0	1:30	50	22	144	167		26			
E-0	1:30	50								
F-0	1:32	52	17	120	62		43			
G-0	1:32	52	19	130		140	20	21	7	
A-1	2:30	110	20	116	110					25
B-1	2:32	112	19	118				32	10	16
C-1	2:35	115	16	122			41			12
D-1	2:36	116	20	123		153	36			12

Table No. 28 con't

Sample No	Time	Δt	Inorganic Carbon mg/l	Total Carbon mg/l	Glucose mg/l	Protein mg/l	Glutamic Acid mg/l	Starch λ=520	Starch λ=680	Ammonia mg/l
E-1	2:38	118	15	54						12
F-1	2:40	120	17	93	38		11			19
G-1	2:42	122	18	104		123	20	5	1	21.6
A-2	3:35	175	19	99	68					
B-2	3:37	177	19	114				18	4	
C-2	3:39	179	17	134			23			
D-2	3:40	180	22	120		153	33			
E-2	3:42	182	16	54						
F-2	3:42	182	17	72	0		6			
G-2	3:44	184	17	93		110		1	0	
A-3	4:30	230	18	73	21					
B-3	4:33	233	18	109				0	3	
C-3	4:35	235	35	111			61			
D-3	4:38	238	22	118		130	26			
E-3	4:40	240	16	55						
F-3	4:40	240	17	62	0		6			
G-3	4:42	242	17	90		95		0	0	
A-4	5:23	383	17	64	0					
B-4	5:25	385	18	109				7	3	
C-4	5:26	386	21	103			45			
D-4	5:28	388	22	114		135	31			
E-4	5:30	390	16	55						
F-4	5:30	390	18	63	0		7			
G-4	5:32	392	17	86		88	22	0	0	
A-5	6:15	435	18	64	16					11
B-5	6:16	436	18	85				6	2	14
C-5	6:17	437	20	72			13			17
D-5	6:19	439	19	87		115	28			14
E-5	6:20	440	15	50						10
F-5	6:22	442	17	59	0		7			13
G-5	6:24	444	13	62		73		0	0	20
Additional Feed Solutions added to each reactor										
A-7	6:42	462	15	134	165					
B-7	6:44	464	14	165				165	132	
C-7	6:45	465	11	131			106			
D-7	6:47	467	20	166		205	66			
E-7	6:50	470	14	51						
F-7	6:52	472	16	93	56		27			
G-7	6:53	473	14	124		100	22	57	25	

Table No. 28 con't

Sample No	Time	Δt	Inorganic Carbon mg/l	Total Carbon mg/l	Glucose mg/l	Protein mg/l	Glutamic Acid mg/l	Starch		Ammonia mg/l
								λ=520	λ=680	
A-8	8:58	598	12	62	27					
B-8	8:59	599	12	136				42	5	
C-8	9:00	600	18	90			85			
D-8	9:01	601	19	120		160	71			
E-8	9:02	602	16	145						
G-8	9:04	604	13	84		81	37	0	0	
A-9	9:45	645	11	51	0					
B-9	9:45	645	10	129				19	5	
C-9	9:46	646	19	75			56			
D-9	9:46	646	19	116		155	59			
E-9	9:47	647	13	43						
G-9	9:47	647	13	77		75	44	0	0	
8-12-70										
A-10	10:15	1395	14	75						
B-10	10:15	1395	13	51						
C-10	10:15	1395	14	47						
D-10	10:15	1395	17	78		105				
E-10	10:15	1395	18	69						
F-10	10:15	1395	12	134						
G-10	10:15	1395	16	64		75				

Footnotes: Suspended solids were determined on the innoculum by millipore filtration and drying and showed 484 and 512 mg/l respectively on repeat tests.

TABLE NO. 29

Effect of Milk Solids Concentration on Rate of Biological Degradation in a Film Flow Reactor

Experiment No. 8-20-69, Plane Test #7, Film Flow Reactor Using Fixed Slime Layer on Inclined Surface

Film flow reactor was acclimated for 3 weeks on dry milk solids feed solution. Growth was spotty, yellowish-beige in color and had a gelatinous surface. Slope of the surface was maintained at 0.0777 inches/24 inches. Samples were collected from the mid section (4.4 in. wide) for analysis.

Sample	Flow Rate ml/min	Carbohydrate Concentration mg/l	Organic Carbon Concentration mg/l	Protein Concentration mg/l
100 mg/l feed solution		38	40	81
P-1	30	3	25	24
P-2	21	0	29	40
P-3	8.3	0	24	30
P-4	1.5	0	16	3
100 mg/l feed solution		47	40	65
PL-1	17	34	27	5
PL-2	8.2	21	24	1
50 mg/l feed solution		18	24	19
PL-3	43	13	18	5
PL-4	25	11	17	5
PL-5	8.1	4	16	5
PL-6	3.5	0	10	2
200 mg/l feed solution			89	139
PL-7	42		60	73
PL-8	26	75	68	132
PL-9	5.6		62	117
PL-10	2.4		51	104
500 mg/l feed solution		162	220	308
PL-11	51	156	195	292
PL-12	18	132	180	243
PL-13	7.3	87	180	266
PL-14	2.4	56	120	139

TABLE NO. 30

Effect of Milk Solids Concentration on Rate of Biological Degradation in a Film Flow Reactor

Experiment No. 9-4-69, Plane Test #8, Film Flow Reactor Using Fixed Slime Layer on Inclined Surface

This is an extension of Experiment #8-20-69, Test #7

Sample No	Flow Rate	Organic Carbon mg/l	Carbohydrate Concentration mg/l	Protein Concentration mg/l
Feed Solution (50mg/l)		28	11	40
P-1	7.0ml/10min	9	4	11
P-2	4.8ml/min	10	5	5
P-3	15ml/min	14	12	10
P-4	28ml/min	15	14	18
200 mg/l feed		78	76	133
P-5	26ml/min	63	41	102
P-6	12ml/min	54	10	65
P-7	3.2ml/min	39	5	87
P-8	3.5ml/10min	15	2	35
125 mg/l feed		50	33	88
P-9	27ml/min	44	1	70
125 mg/l feed		51	11	88
P-10	7.6ml/10min	12	3	10
P-11	6.3ml/min	25	10	23
P-12	13ml/min	31	8	32
P-13	23ml/min	40	8	58

TABLE NO. 31

Effect of Lauric Acid Concentration and Mixed Feed Solutions on Rate of Biological Degradation in a Film Flow Reactor

Experiment No. 3-17-70, Plane Test #9, Film Flow Reactor using Fixed Slime Layer on Inclined Surface

Film flow reactor was acclimated to a 50 mg/l lauric acid feed solution for a period of several days. The slope of the plane was maintained at 0.0782 inches per 24 inches. Samples were collected from the center portion 4.4 in. width and analyzed for organic carbon. Initial part of test used only lauric acid feed. Mixed feed solutions were used at the end of the test as shown.

Sample	Feed Rate ml/min	Organic Carbon mg/l	Glucose mg/l	Glutamic Acid mg/l
100 mg/l lauric acid		69		
P-1	60ml/5min	52		
P-2	12.1ml/min	67		
P-3	25ml/min	64		
100 mg/l lauric acid		67		
P-1	22.3ml/min	63		
P-2	12ml/min	61		
P-3	12.1ml/5min	46		
P-4	57ml/min	66		
P-5	41.0ml/min	65		
P-6	21.0ml/5min	51		
P-7	22.1ml/5min	54		
50 mg/l lauric acid		42		
P-1	29ml/min	38		
P-2	18.1ml/min	38		
P-3	11.9ml/min	35		
P-4	11ml/min	33		
P-5	10.1ml/5min	28		
P-6	15.3ml/2min	33		
P-7	11.0ml/5min	28		
P-8	10ml/10min	21		
25 mg/l feed		21		
P-1	47ml/min	18		
P-2	34.2ml/min	17		
P-3	44ml/2min	18		
P-4	25.0ml/2min	17		
P-5	37.0ml/6min	16		
P-6	16.4ml/5min	10		
P-7	17.0ml/10min	7		
100 mg/l mixed feed (glucose, glutamic acid, Lauric acid)			71	130

Table No. 31 con't

Sample	Feed Rate ml/min	Organic Carbon mg/l	Glucose mg/l	Glutamic Acid mg/l
P-1	61ml/min	140	72	126
P-2	47.0ml/min	138	72	132
P-3	38.0ml/min	134	71	127
P-4	25.5ml/min	134	72	131
P-5	17.3ml/min	138	76	140
P-6	20.0ml/2min	132	72	114
P-7	30.5ml/4min	128	75	123
100 mg/l	mixed feed	144	75	109
P-1	51.0ml/min	70	75	114
P-2	43.8ml/min	69	74	113
P-3	33.0ml/min	71	75	130
P-4	27.0ml/min	71	76	126
P-5	20.4ml/min	72	75	120
P-6	16.8ml/min	70	73	121
P-7	17.2ml/2min	69	72	117
P-8	19.6ml/4min	67	69	97
P-9	5.8ml/20min	48	45	24

TABLE NO. 32

Effect of Flow Rate on Biological Degradation of Mixed Feeds in a Film Flow Reactor

Experiment No. 4-9-70, Plane Test #10, Film Flow Reactor Using a Fixed Slime Layer on an Inclined Surface

Film flow reactor was acclimated using mixed feed (glucose, glutamic acid and lauric acid) for a period of 5 days. Flow was interrupted for one day on 4/5/70. The slope of the plane was maintained at 0.0782 inches per 24 inches. Samples were collected from the center section (4.4 in. wide) for analysis.

Sample	Feed Rate	Organic Carbon mg/l	Glutamic Acid mg/l	Glucose mg/l
Feed Solution		136	68	71
P-1	36ml/min	134	89	71
P-2	29.8ml/min	132	85	70
P-3	25.4ml/min	132	79	71
P-4	33.0ml/2min	130	83	71
P-5	18.0ml/2min	128	81	73
P-6	16ml/2.5min	120	74	73
P-7	9.0ml/3min	106	75	66
P-8	6.3ml/7min	96	81	61

Continuation 4-10-70, Plane Test #11, 4/16/70

Sample	Feed Rate	Organic Carbon mg/l	Glutamic Acid mg/l	Glucose mg/l
Feed Solution		124		69
P-1	49ml/min	120		89
P-2	35.9ml/min	114		84
P-3	21.0ml/min	116		82
P-4	24.1ml/min	108		79
P-5	36.9ml/2min	102		70
P-6	24.8ml/2min	98		64
P-7	16.1ml/3min	95		72

TABLE NO. 33

Evaluation of Mixed Feed Solutions at Various Flow Rates in a Film Reactor

Experiment No. 8-20-70, Plane Test #13, Film Flow Reactor Using A Fixed Slime Layer on a Inclined Surface

The film flow reactor was acclimated to a mixed feed of glucose, glutamic acid and lauric acid. Ammonia addition was limited in order to avoid interference with amino acid test. The slope of the plane was maintained at 0.0782 inches per 24 inches. Samples were collected from the center reaction (4.4 in. wide) for analysis.

Sample	Feed Rate ml/min	Organic Carbon mg/l	Glucose mg/l	Glutamic Acid mg/l
Feed Solution		140	138	102
P-1	11.4	124	111	102
P-2	7.3	120	92	95
P-3	3.7	98	62	98
P-4	8.8	120	75	90
P-5	22.	130	116	93
P-6	28.6	130	77	96
P-7	40.	128	118	63
	Continuation - Plane Test #14			
Feed Solution		78	59	50
P-1	100	71	58	45
P-2	78	73	55	44
P-3	60	76	54	87.5
P-4	36	74	54	47.5
P-5	36	76	54	50
P-6	23	74	50	55
P-7	20	76	54	56
P-8	17	76	45	55
P-9	9	71	40	56
P-10	7.5	73	35	56
P-11	3.8	66	28	46

TABLE NO. 34

Evaluation of Starch, Protein and Lauric Acid Feed Mixtures in a Film Reactor

Experiment No. 8-29-70, Plane Test #15, Film Flow Reactor Using a Fixed Slime Layer on an Inclined Surface

The film flow reactor was acclimated to a mixed feed of 100 mg/l starch and protein and 50 mg/l of lauric acid feeds. The slope of the plane was maintained at 0.0782 inches/24inches. Samples were collected from the center reaction (4.4 in. wide) for analysis.

Sample	Feed Rate ml/min	Starch, mg/l λ =520	λ =680	Organic Carbon mg/l	Peptone mg/l
Feed Solution		104	99	54	118
P-1	51	104	93	55	143
P-2	33	76	72	53	118
P-3	20.3	76	66	54	118
P-4	13.	60	48	53	108
P-5	8.	58	38	53	115
P-6	5.	33	15	52	118